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An Investigation of the Behaviour of Pectic Materials in Apples and other Plant Tissues.[1]

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With Plates XII–XIV and nine Figures in the Text.

CONTENTS.

[1] The senior author (A. S. H.) is responsible for the greater portion of the microscopical work.

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Introduction.

During the last few years a purely chemical investigation of the pectic constituents of apples has been carried out in these laboratories (9, 10, 11, 12). The evidence from this chemical study indicates that the pectic constituents occur in the apple in at least three forms: (1) *pectin*, a water-soluble substance which develops in the tissues during ripening; (2) *pectose*, an insoluble compound located in the cell-wall which, as ripening proceeds, appears to give rise to soluble pectin; (3) a complex containing pectic acid or a salt of pectic acid, of which complex the middle lamella is either partially or entirely composed. These three constituents have been extracted fractionally and estimated individually, and their relative proportions in the tissues determined.

During the progress of the chemical work, the value of a parallel microscopical study of the pectic changes in the cell-wall of the apple became evident. The results of such a microscopical[1] and micro-chemical investigation are here put forward, and an attempt has also been made to correlate the results obtained with those accruing from the purely chemical work.

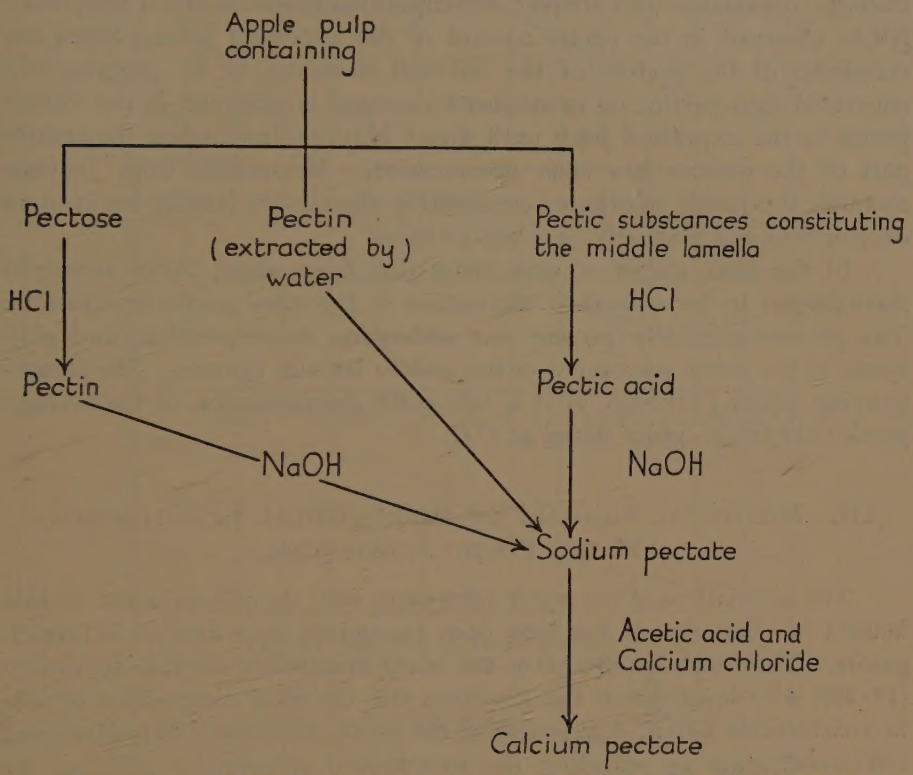
A brief statement will first be given of the results of the purely chemical study of the pectic changes, together with a brief description of the methods employed.

II. Pectic Changes as studied by Chemical Methods.

The chemical methods[1] depend upon the fact that the pectic compounds can be converted into an insoluble calcium salt of pectic acid of constant composition. A known weight of apple pulp is taken, and the soluble pectin is washed out with cold water and estimated as calcium pectate. The pulp is then treated with hydrochloric acid, and the pectose converted, presumably by a process of hydrolysis, into soluble pectin which is washed out as before and estimated. The residue is then treated to remove the remaining pectic substances constituting the middle lamella. The acid

[1] For fuller details see Carré (10, 11, 12).

treatment for removing the pectose simultaneously decomposes the middle lamella into free pectic acid. Pectic acid, however, is only partially soluble in dilute acid and in water, and cannot be extracted entirely as such from the tissues. By warming with dilute caustic soda, the pectic acid is con-



TEXT-FIG. 1. Diagram illustrating the extraction and estimation of pectic compounds.

verted into the water-soluble sodium salt, which may be removed from the pulp by washing and estimated by converting into the calcium salt. The accompanying diagram (Text-fig. 1) illustrates the processes of extraction[1] and estimation of the various pectic compounds in apple tissue.

These chemical methods were employed in tracing the changes in the pectic constituents of the apple from the stage of early ripening to the last stages of senescence and death, a period extending from September to the end of June in the following year, the fruit being held in cold storage at 1° C.

It was observed that, as ripening proceeds, there is a tendency towards

[1] Ammonium oxalate has been used by many authors, Mangin (38–44), Schryver and Haynes (52), &c., as a solvent for pectic substances. This reagent, however, removes all the various pectic substances. For this reason the use of this reagent was abandoned, since at the time it was desired to trace the relationship between the different pectic components.

degradation and solution of the pectic compounds in the tissues. Soluble pectin is developed in the juice during ripening at the expense of the insoluble pectose of the cell-wall, which exhibits a corresponding decrease in amount. As the fruit becomes soft and over-ripe, the pectin tends to undergo conversion into simpler decomposition products, and a temporary fall is observed in the pectin content of the expressed juice. Since the remainder of the pectose of the cell-wall continues to be progressively converted into pectin, no pronounced decrease is observed in the soluble pectin in the expressed juice until about May or June, when the greater part of the pectose has been decomposed. Meanwhile, from January onwards the pectic substances constituting the middle lamella begin to be decomposed and gradually pass into solution.

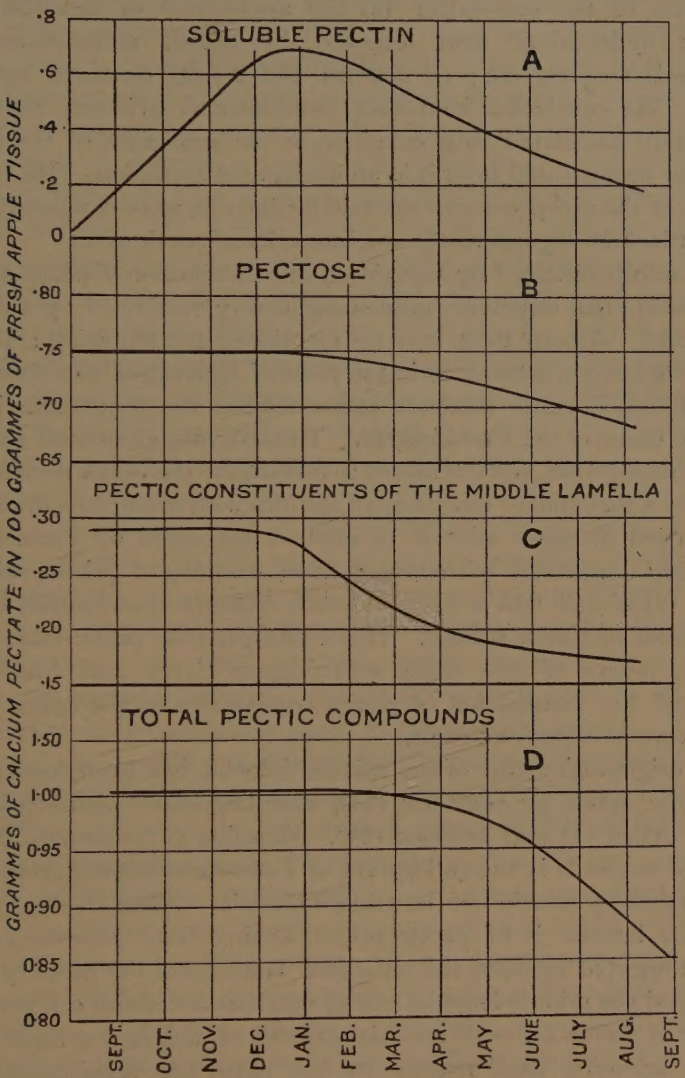
In the later stages of senescence (i. e. from about April onwards) there begins to be a marked diminution in the total pectic constituents. The pectose originally present has undergone decomposition, and only traces of the pectic substances of the middle lamella remain. The accompanying graph (Text-fig. 2) is a schematic representation of the average pectic changes in apples stored at 1° C.

III. Historical Summary of Microscopical Investigations on the Pectic Substances.

The associations of the pectic substances with the cell-walls and middle lamella of plant tissues has long been recognized by a number of investigators. The most important of the early researches are due to Frémy (17–20), who investigated the structure and chemical composition of cells in considerable detail, demonstrating the pectic substances as fundamental cell constituents by effecting the preliminary removal of cellulose by Schweitzer's reagent, and identifying the residual cell framework with substances of pectic nature by chemical tests—such as solution by acids and alkalis. Frémy first pointed out the presence of an insoluble pectic compound—pectose—in fruits which gives rise on ripening to a water-soluble modification—pectin. Mulder (46) considered cells to be united by a kind of cell cement, 'inkrustierenden Substanz', which he showed to be of pectic nature, and regarded the cell-walls themselves as an intimate mixture of cellulose and pectose. Payen (49), Harting (26), Kabsch (34), Vogl (60), and later, Wiesner (65) and Tschirch (55) advanced similar views that pectic substances were exclusively confined to the middle lamella regions of cell tissues, of which they were the main constituents in the form of potassium or calcium pectates.

During the years 1889–93, Mangin published his important series of researches on the detection of pectic substances and their distribution in the soft tissues of numerous plants. Mangin accepts the hypothesis put forward

by Frémy, that the production of pectin is an important factor in the ripening of fruit, and considers that the development of the fruit is asso-



TEXT-FIG. 2. Graphical representation of the pectic changes in apples from the time of picking to the last stages of senescence.[1]

ciated with fundamental changes in the pectic constituents. Mangin makes some attempt to locate the different pectic structures in various plants, basing his investigations on a combination of two methods: (1) the use of

[1] The chemical data are not complete, since they were only begun after the apple had been picked (i. e. after the period of full development), whereas the microscopical features were followed from the flowering period onwards; see Tables I and II.

basic stains such as safranin, methylene blue, and in his later work, ruthenium red, in order to differentiate the pectic compounds from the other constituents of the cell-walls; (2) the application of chemical reagents (alcoholic hydrochloric acid followed by alkali, ammonium oxalate, Schweitzer's reagent, &c.) to confirm the results obtained by staining methods. He concluded from such experimental evidence that pectose is intimately associated with cellulose in the substance of the cell-walls, and cannot be separated from it in an unchanged condition. He found that the lining of the air-spaces and the middle lamella were composed of pectic material which he regards as the calcium salts of pectic acid.

The results obtained by Mangin on the distribution of pectic substances in specialized plant structures were subsequently confirmed by a number of investigators. Among these may be mentioned the following: Vidal (59), who detected pectic substances in the roots of *Equisetum*, and Bancroft (24) who modified Mangin's methods and examined the pectic constituents in the xylem tissue of the *Pteridophyta*. Tunman (56) examined the roots of various Umbelliferae, and attempted to investigate the pectic metamorphoses of fruits by a preliminary treatment of sections with methylene blue, followed by immersion in sugar solution (a method suggested by Tschirch for the solution and separation of modified from unchanged pectic substances). Sampson (51) found that a series of pectic changes accompanied abscission in the leaves of *Coleus Blumei*. Howe (30) detected pectic material in the outer cell layers of root hairs, and Tupper-Carey and Priestley (57) investigated the distribution of pectic compounds in the apical meristem tissue of various stems and roots.

The origin and nature of the middle lamella has been investigated in considerable detail by Gardiner (21), and important contributions were made by Allen (1) and Devaux (14). Gardiner (21) describes the partial solution of the walls in the endosperm of *Tamus communis* during germination preceded by solution of the middle lamella. Allen (1) suggested that the middle lamella is of pectic origin, arising from secondary products which are inserted between the separated lamellae of the primary cell-wall. He regarded the middle lamella, not so much as a definite cell cement, but as a layer of plastic cell-wall material capable of undergoing rapid modification, and adapting itself readily to the particular requirements of the tissues.

Devaux (14) disputed the view that the composition of the middle lamella is exclusively calcium pectate, but inclined to the view that the pectic substances of plant tissues are all closely akin to pectose, being different members of a group of wall constituents. He agreed that calcium exists in the middle lamella, but pointed out the lack of evidence that it is in combination with pectic acid, or indeed that pectic acid exists as such at all in the middle lamella.

Hence there are varying opinions as to whether pectose or pectic acid constitutes the middle lamella of plant tissues. In the opinion of the writers, based on their investigations, it is unlikely that the middle lamella is composed of either pectose or pectic acid, but of a far more complex pectic substance, possibly containing residues of both pectose and pectic acid.

The significance of the pectic compounds in relation to bacterial and fungal attack, especially in connexion with the retting of flax, &c., has also been submitted to detailed investigation in the past. Van Tieghem (53) first showed definitely that bacterial agents were responsible for natural retting of textiles. He attributes the process to *Bacillus amylobacter* (*Clostridium butyricum*) an anaerobic organism which becomes active when rotting ensues in plant tissues, and whose prolonged action results in the isolation of the cellulose fibres. Later, Winogradsky (64), in conjunction with Fribes, pointed out that the retting of textile fibres was due to decomposition of the pectic cell cement by bacteria, and attributes this so-called 'pectic fermentation' to enzymes secreted by the bacteria themselves. Behrens (4) found that *Clostridium* was the chief agent in commercial retting and that numerous bacteria could effect it. He found that no action occurred on the cellulose, and compares the process of pectic disturbance to the changes induced by the chemical agencies employed by Mangin.

Meanwhile the relationship of pectic substances to fungal attack had received much attention. De Bary (3) observed that the fungus *Peziza sclerotiorum* caused the death of plant tissues by secreting a substance which effected partial disintegration of the cell-walls and which he attributes to an enzyme in the digestive juices of the invading fungus. Extracts of the diseased tissue caused a similar disintegration of the healthy tissue of carrots, but if previously boiled before inoculation no effect was produced. Marshall Ward (61) observed that various species of *Botrytis* have a similar effect on healthy plant tissue. Harding and Morse (25) and Jones (32), in a series of investigations on the soft rot of carrots and other vegetables, found that the attack of the bacillus involved solution of the middle lamella with the consequent separation of the cells in the areas of the invaded tissue. From an examination of the numerous bacteria producing soft rots, Jones concludes that a cytolytic enzyme (pectinase) is produced, capable of causing middle lamella solution. Cooley (13) and Hawkins (24), however, found that fungal disease does not invariably involve middle lamella solution, since the fungus *Sclerotinia cinerea*, (Bon.) Schroter, causing brown rots of plums and peaches, does not invade the middle lamella region of the tissues and does not apparently make use of pectin. Brown (8) obtained a powerful enzyme extract from the germ tubes of *Botrytis cinerea* and found that the action of this extract on various plant tissues (potato, turnip, beet, apple, &c.) operated in three stages, according to the time of action: (1) solution of the middle lamella, (2) *partial* disintegration of the cell-

walls, (3) death of the cells. In no case did Brown observe complete solution of the cell-wall, which observation is readily interpreted by assuming that the enzyme is only capable of acting on the pectic compounds of the cell-wall and middle lamella, and not on the cellulose components of the tissues. Confirmation of Brown's work is afforded by Valleau (58) in a series of investigations on the mode of attack of *Sclerotinia cinerea*. Valleau also attributes the solvent action to the secretion of an enzyme—the cytase or pectinase of other workers—and shows by a series of photomicrographs that the enzyme is secreted by the fungal hyphae in advance of the invading fungus. Willaman (62), also working on *Sclerotinia cinerea*, showed that if the fungus were grown on pectin as its sole source of nutriment, the pectin was converted into an insoluble gel of pectic acid, and that, ultimately, reducing sugars were split off from the pectic acid and assimilated by the fungus. Harter and Weimer (63) have obtained similar results with different species of *Rhizopus* and *Botrytis cinerea*, and find that the maximum macerating effect is observed when the capacity for acid production by the organism attains a pH value between 3·0 and 4·0.

None of these microscopical studies of the pectic substances, however, deal with their possible significance in the various phases of plant life—such as growth and development, the ripening of fruits, and the various stages accompanying senescence of plant tissues.

IV. Microscopical Methods for the Detection of the Pectic Substances.

(a) *Application of Staining Reagents.* The basic stains, such as naphthalene blue, methylene blue, and safranin, recommended by previous investigators give unreliable results, since they do not exhibit specific affinity for pectic substances. On the other hand ruthenium red, a compound investigated by Joly (31) and employed with success by Mangin (45) for pectic mucilages, has proved entirely satisfactory. It does not stain pure cellulose, and possesses more specific affinity for pectic substances than any other stain used in botanical microtechnique. For these reasons ruthenium red has been employed in the course of this investigation for detecting the presence of pectic substances in plant tissues.[1]

Gums, mucilages (45, 56), fatty acids (57), and gelose (56) have been recorded also as staining with ruthenium red, but the presence of these substances has not been detected in apples, and in tissues where they do occur

[1] The vascular tissue in apples is found to stain very strongly with ruthenium red, but it s probable that the staining is due to adsorption phenomena and does not necessarily denote the presence of pectic compounds, since it is unaffected by the application of the usual solvents for pectic substances. Also the vascular tissues in apple residues from which all substances of pectic nature have been previously removed, are observed to stain as intensely as before such treatment.

the pectic substances are readily distinguishable from them by characteristic chemical reactions (see Section VI).

In all cases freshly prepared hand sections were used for the tests, and serial sections were cut from a radial cylinder of the tissue in order to take into account variation in the individual apple. The variation in different samples consisting of ten individuals each of the same variety of apple was also observed.

(b) *Application of Various Pectic Solvents.* It has been found possible to recognize the structures in the apple tissue which stain with ruthenium red as substances of a pectic nature by the application of chemical reagents known to effect the removal of the pectic constituents of the tissues (see Section II). Ammonium oxalate is useful for this purpose, since it readily dissolves out all the pectic compounds, leaving the cellulose unaltered. Careful treatment of the sections with hydrochloric acid followed by potassium hydroxide also effects the removal of these compounds, the use of these reagents being an adaptation of the chemical methods already described. The degree of alteration induced by this treatment depends upon the concentration and the time of action of the reagents. Prolonged action results in the more or less complete removal of the pectic compounds, and it was observed that the cell-walls no longer stain with ruthenium red and that the cells become separated from one another owing to the solution of the middle lamella.

(c) *Application of Cellulose Solvents.* The use of the purely chemical and microscopical methods described above confirms the view that the cellulose of the cell-walls is intimately associated with pectose, and that the middle lamella may be conceived as a kind of cell cement, composed of a complex containing pectic acid or pectates, which encases and binds together the component cells. This conception of the distribution of the pectic compounds in the tissues is supported by the evidence obtained from the use of the reverse process of dissolving out the cellulose and examining the disposition of the remaining cell-wall substances. Sections were treated with Schweitzer's reagent, according to a method first described by Frémy (17–20) and later developed by Mangin (38–45), which removes the cellulose and leaves the pectic substances in an insoluble condition in the tissues, thereby maintaining the original framework. After this treatment the sections are extremely fragile, but microscopical examination shows that the structural appearance of the tissues is unaltered, the outline of the cells being maintained by the presence of the pectic constituents. Ruthenium red stains this residual framework deeply, and subsequent treatment with pectic solvents (ammonium oxalate, or hydrochloric acid followed by weak alkalis) gradually dissolves away the framework, leaving no visible sign of the original structures.

The authors have found that a combination of staining methods and

the application of chemical reagents is entirely satisfactory for the examination of the disposition of the pectic substances in plant tissues and of the changes which they undergo, and have adopted these methods consistently throughout this investigation.

V. Pectic Changes during the Growth and Senescence of Normal Apples and Pears as observed by Microscopical Methods.

(a) *From the Flowering Period onwards until the Apple has attained its Maximum Size.*

In the young fruit the tissue is compact, the parenchymatous cells exhibit regular outlines, and the intercellular spaces are exceedingly minute (Pl. XII, Fig. 1). As development proceeds, the cells become irregular in shape and size, the cell-walls become thinner, and the intercellular spaces are enlarged. At first the cell-walls are uniformly stained with ruthenium red, indicating the even distribution of pectic substances throughout the walls, but at quite an early date (June) the walls begin to exhibit unequal staining (Pl. XII, Figs. 2–4), the portions of the wall abutting on the intercellular spaces being more strongly stained than those in contact with other cells. For convenience in description, the portions of the wall of a given cell *in contact with* and *free from* respectively another cell will be termed 'walls of contact' and 'free walls' in the course of this paper. As ripening proceeds, more pronounced changes are evident, of which full details are given in chronological order in Table I.

At the close of this developmental stage the middle lamella consists of an extremely thin layer of pectic material, which is not easily distinguishable from the remaining cell-wall substance. The disposition and the forms assumed by the other pectic constituents of the cell-wall (Pl. XII, Fig. 5) are as follows:

(1) *Discs.* These structures are extremely small aggregations of pectic material formed during cell-growth. They vary in number and size (2–8 μ), and are distributed irregularly in the walls of contact. In surface view they present a more or less elliptical or circular form, and sometimes the outline is irregular. When fully developed they are not uniformly stained, the surface exhibiting a finely granular or alveolar appearance. In sectional view they are observed as short rods of stainable material, and the discs situated in the wall of contact of a particular cell stand opposite those present in the neighbouring wall of contact of the cell to which it is attached. (2) *Crescents.* Aggregations of pectic substance staining deeply with ruthenium red. These structures are usually larger than the discs and are disposed as a rule near the boundary of a wall of contact. In surface

view they present a crescentic form with the horns of the crescent directed towards the centre of the wall. The crescents do not exhibit any obvious structural details. In a sectional view their appearance varies with the

TABLE I.

Details of the Pectic Changes observed during the Development of the Bramley's Seedling Apple in 1924.

Date.	*Dimensions of the Apple in cm.*	*Dimensions of the Cells in μ*[1].	*Dimensions of Air-spaces in μ.*[1]	*Microscopical and Chemical Observations.*
	Diam. Ht.			
May 27	0·9 × 1·1	38 × 38	36 × 12	Tissues compact, numerous thin partition walls present.
June 12	1·8 × 1·5	90 × 67	114 × 36	Cell-walls show signs of unequal staining.
June 24	3·5 × 3·0	118 × 85	203 × 107	Unequal staining more pronounced. The stained portions finely granular.
July 2	4·3 × 5·5	227 × 136	213 × 80	Minute feebly stained areas (*discs*)[3] present in the walls of contact. A little starch present.
July 16	6·0 × 5·0	186 × 135	366 × 135	The walls of contact are bounded by a narrow band of pectic substance. Much starch. Parchment layer lining the ovarian cavity stains with phloroglucin.
July 25	6·5 × 4·7	—	—	*Bands*[3] more prominent up to 8 μ wide.
Aug. 8[2]	6·7 × 6·0	—	—	Minute extracellular projections (*papillae*)[3] observed. Traces of pectin appear in the expressed juice.
Aug. 14	7·0 × 5·5	—	—	*Papillae*[3] more numerous. Traces of pectin appear in the expressed juice.
Sept. 3	8·0 × 5·5	—	—	*Bands*[3] less prominent. Small crescentic bodies (*crescents*)[3] present in the bands, but of rare occurrence. Slight increase in pectin in the expressed juice.
Sept. 17	7·0 × 6·0	—	—	Starch feebly developed. Appreciable amounts of pectin present in the expressed juice.
Oct. 25	8·5 × 6·0	248 × 190·4	—	*Bands*[3] feebly developed. *Crescents*[3] and *discs*[3] distinct and fairly numerous. Starch absent.

particular angle of inclination of the wall under observation. (3) *Bands.* Thin bands of pectic substance which occur at the boundaries of walls of contact. At maturity, if present, the bands are usually only feebly stained with ruthenium red. (4) *Papillae and small globules.* Minute extracellular projections with which the free walls of the cells are studded. These

[1] In arriving at these dimensions the average of not less than twenty of the largest of the cells or intercellular spaces observed in one or more sections is given in each case.

[2] Systematic chemical investigations were begun about this date.

[3] For convenience in description the pectic structures described above will be referred to respectively as discs, bands, papillae, and crescents, in the course of this communication.

structures stain feebly with ruthenium red. The papillae may be regarded as initial stages in the formation of the small globules, which are developed in great abundance during the late stages of senescence.

The observations recorded in Table I indicate that during the development of the cells and intercellular spaces the pectic substances undergo a series of gradual changes. The discs, crescents, and extracellular projections do not develop simultaneously, and the appearance of the crescents is preceded by the peculiar bands of pectic substances already described. These bands are often absent when the apples are fully matured and the crescents are well developed, and it will be shown subsequently that they are again manifest when the crescents are undergoing further changes during the period of senescence.

The varieties Bramley's Seedling, Cox's Orange Pippin, and Worcester Pearmain exhibit similar pectic changes during the development of the apple. With the Worcester Pearmain apples the development of the various pectic substances takes place somewhat earlier than with the Bramley's Seedling; this difference is probably related to the characteristics of the classes of apples which these varieties represent. The Worcester Pearmain ripens relatively early, but does not keep well. Bramley's Seedling, on the other hand, is a late variety and possesses good keeping qualities.

Since the cell-wall is extremely thin, it has not been possible to determine accurately by the technique employed in this investigation whether the crescents and discs are situated in the inner layer of the wall or middle lamella region. It is regarded as probable that they bear some relation to the pectic changes occurring in both the inner and outer wall regions.

In attempting an interpretation of the appearance and changes undergone by the pectic substances described above, it may be suggested that they are due to the expansion of the cells. During the building up and growth of the cell-wall, the uniform layer of pectic material which was primarily laid down in the young cell-wall must become spread over a larger area as the cells enlarge, since preliminary chemical analysis shows that there is no appreciable increase in the amount of pectic material in the cell-wall during this period of growth. The pectic constituents thus form an increasingly thinner layer, which may be conceived to give rise either to the disconnected areas seen in the adult tissue as unevenly staining regions in the cell-wall, or possibly contribute to the disc-like structures which occur in the middle lamella region of walls which are in contact with one another.

It is important also to note in interpretation of the facts observed that throughout this period the development of the intercellular spaces involves modification of the middle lamella, which is due to the partial mechanical

separation of the cells. During the primary development of the air-spaces globules are not observed, but it will be shown subsequently that they appear later and are associated with secondary modifications of the intercellular spaces.

It may be concluded from these observations on the redistribution of the pectic substances during growth, that they constitute a plastic layer capable of considerable modification and adaptation as development of the tissues proceeds.

(b) *From the Preceding Stage until the Onset of Physiological Break-down.*

This stage has been studied chiefly in apples kept under low temperature conditions. It is marked by a slow modification in form of the various structures constituting the pectic framework, culminating, when physiological break-down ensues, in their complete disappearance. Stability to pectic reagents decreases gradually throughout this stage of development, the pectic structures being readily brought into solution.

Before embarking on this study of changes during senescence, the question of the variability of the pectic substances, that is to say, variation in time of the appearance of different stages of pectic transformation, was studied in considerable detail. The probable range of the variation in the individual apple was thus ascertained. A certain amount of variation was found on comparing sections taken from the inner and outer regions of the flesh, from different sides, and from the upper and lower portions of the individual apple. The variation in degree of pectic change shown by an individual apple is represented diagrammatically in Text-fig. 3 (facing p. 210), where the letters *a–h* denote different stages in the transformation of the crescentic bodies (see Pl. XII, Figs. 6–14). The examination of individual apples during senescence shows that the stability of the pectic substances (papillae and small globules excepted) is least affected in the inner regions of the tissues; they are, in fact, observed to become progressively more unstable in a radial direction outwards, the maximum change being detected in the periphery. This indication of more advanced change in the pectic constituents situated in the peripheral flesh of the apple may be readily attributed to the fact that this tissue region is nearer the external air than the more deeply seated tissue, and would be directly affected by factors (light, temperature, &c.) which affect the process of ripening.

The variation existing in a sample consisting of a number of apples was also ascertained. Generally speaking, the least variation is found at the commencement of the period of senescence. The crescents and discs are more or less uniformly distributed, and usually well developed, while the middle lamella as yet shows no marked signs of alteration.

During senescence variation increases, and the difference in the degree of pectic change observed when different parts of the same apple or different apples from an average sample are compared becomes more and more evident.

Owing to this variability in different parts of the same apple and in different apples, the method of investigating pectic changes was standardized. Radial cylinders of tissue were taken by means of a cork borer from different sides of the apple. From each cylinder several tangential sections were made at each of three different positions chosen at distances of approximately 0·5, 1·5, and 2·5 cm. respectively from the skin. This process was carried out on representative samples of apples obtained at regular intervals from the Low Temperature Research Station at Cambridge.

The changes which the pectic substances undergo during the period of full maturity and senescence may be outlined briefly as follows:

(*a*) The discs (Pl. XII, Figs. 6–14) exhibit structural alteration and become swollen (Pl. XII, Figs. 9–11). Later, they show a marked diminution in their capacity for staining with ruthenium red (Pl. XII, Fig. 12), and finally disappear (Pl. XII, Figs. 13 and 14).

(*b*) The crescents (Pl. XII, Figs. 6–14) also undergo a definite sequence of changes. At first they become surrounded with diffuse stainable material (Pl. XII, Fig. 7). Later the stainable material surrounding neighbouring crescents coalesces, forming a continuous band (Pl. XII, Figs. 8 and 9), which follows the boundary of a wall of contact. Meanwhile the crescents appear to be losing substance (Pl. XII, Fig. 9), and when the bands are prominently developed, only the outlines of the crescents remain (Pl. XII, Figs. 10 and 11). Finally, when the areas undergoing solution abut on an intercellular space, large globules are formed which project from the bands into the intercellular space (Pl. XII, Figs. 10 and 11). In the later stages complete disappearance of the bands and globules is observed (Pl. XII, Figs. 13 and 14).

These changes in both crescents and discs bear the interpretation that pectose is steadily breaking down to produce soluble derivatives—pectinic acids and ultimately pectin. Pectin in its turn is gradually decomposed into other soluble bodies, sugar, organic acids, &c., which no longer exhibit the typical staining reactions of the pectic complex. This progressive decomposition accounts for the altered appearance of the discs and crescents and for the diffuse pectic material which surrounds the latter. These structures may be supposed to absorb water from the intercellular passages, and possibly from the cell contents,[1] thereby producing the phenomena of swelling, gradual solution, and finally globule formation as described above.

[1] Decomposition of the pectic constituents of the cell-wall will tend to facilitate the outward passage of water.

The fact that it is possible to reproduce such phenomena artificially, and that ready solution of the diffuse material referred to can be brought about by irrigation or soaking sections in water, supports the above-mentioned interpretation of the changes under discussion. It may be pointed out also that the pectose in the cell-wall is much less stable towards reagents at this stage than it is in the earlier stages of development.

(*c*) The papillae and small globules increase in size and number during this phase, and are more readily stained with ruthenium red than before (Pl. XII, Fig. 5).

The development of these bodies may be attributed also to the steady production of pectin and its tendency to absorb and dissolve in any water which may be in contact with it. The increase in staining capacity of the globules would necessarily follow from increase in the size and concentration of their substance as the process of pectose conversion continues.

(*d*) The cells tend to separate from one another, assuming a bottle-like form, and minute aggregations of pectic material are left in the separated walls (Pl. XIII, Fig. 24, and Pl. XIV, Fig. 25). This suggests that decomposition of the middle lamella pectic substances has now set in, resulting in the initial stages of cell separation.

The period of full ripeness is characterized by further microscopical changes. The various pectic structures associated with the cell-wall become diminished in size or disappear altogether, the cell-walls appear thin and transparent, and the tissues stain very feebly as a result of the decomposition of their pectic components (see Pl. XII, Fig. 14). The middle lamella undergoes further decomposition, resulting in a soft condition of the apple, as the cells tend to separate from one another. In over-ripe fruit and in the last stages of senescence, these features become more pronounced. The cells separate from one another with slight mechanical pressure, or on immersing the sections in water. The spaces between the separated cells are often occupied by a stainable matter of stringy consistence, presumably consisting of masses of semi-soluble pectic material derived from the disintegrated cell-walls and middle lamella (Pl. XIV, Fig. 32). The majority of the separated cells are dead, as judged by their plasmolysed appearance and altered refractive index (Pl. XIII, Fig. 24, and Pl. XIV, Fig. 25). The cell-wall appears thin and wrinkled, and the protoplasm exhibits a yellow colour.

The accompanying table (Table II) gives a summary of the pectic changes which were recorded during the ripening and senescence of Bramley's Seedling apples stored at 1° C. for a period of eight months.

With reference to Table II the following points are of special interest: (1) During the period from October until the beginning of December, when the first records appearing in Table II were made, the pectic structures in the tissues exhibited little change. Owing to the incidence of 'internal break-

down' in the store from April onwards, the number of apples available for microscopical study was very limited, and it is therefore doubtful whether the surviving samples actually show normal senescent changes. In the previous year (1922–3) 'internal break-down' was less prevalent in Bramley's Seedling apples held under similar storage conditions, and the final stages of normal senescence were reached in average samples in July (1923). Accordingly, the observations made in July, 1923, are given in addition to those of the following year. In 1923 the final stage of senescence in average samples was marked by the concurrent dissolution of the various pectic constituents (globules, cell-wall pectic substances, middle lamella, &c.). The apples exhibited the condition known to the trade as 'mealiness', and the constituent cells of the sections were readily separated by applying slight pressure. A little later, sections showed more or less complete separation of the cells, and the loosened cells exhibited the characteristic bottle-like shapes. In a large number of cases marked plasmolysis of the cell contents was observed also.

In 1924, however, the fruit surviving 'internal break-down' appeared to be more resistant to physiological break-down than the average samples examined in 1923. A general diminution of the pectic substances associated with the cell-walls had taken place before solution of the middle lamella became pronounced, and the condition of 'mealiness' was less evident. Nevertheless, by gentle pressure the cells were readily forced apart, and by careful manipulation with needles it was found possible to change the shape of the individual cells, and to draw them out into the bottle-shaped forms characteristic of the 1923 samples. It seems likely, therefore, that if the stock of apples in 1924 had not become thus depleted, the average samples would have exhibited the phenomena recorded in 1923.

(2) An inspection of the table shows that the factors operating during senescence do not produce simultaneously the same degree of change in the various pectic substances. For example, at a time when the crescents had undergone considerable modification (January 10), the cohesion of the cells was not affected to any marked extent. A similar lack of uniformity in behaviour at a given time was noted in the changes affecting the crescents and discs and papillae respectively. Results obtained from the purely chemical side of this investigation suggest a feasible explanation of these observed facts, in that the various bodies show constitutional variation which is obviously correlated to differences in their susceptibility to the chemical changes obtaining in the maturing fruit.

It will be of interest to compare the changes recorded in Table II with the pectic changes as observed by chemical methods carried out concurrently on similar samples of apples. Chemical methods show that during ripening the tissues gradually become depleted of pectose as it is converted

into the form of soluble pectin. The period of full ripeness is characterized by changes in all three of the pectic constituents, pectose, pectin, and the middle lamella pectic substances. The rate of pectose decomposition is

TABLE II.

Pectic Changes observed in Bramley's Seedling during Storage at 1° C.

Date.	*Crescents.*	*Discs.*	*Papillae and Small Globules.*	*Middle Lamella Pectic Substances.*	*Cell, Shape, Staining, &c.*
1923. Dec. 4	*Crescents* little altered, *bands* feebly developed, *large globules* absent.	Not prominent, distinct on boiling (H_2O).	None observed.	Unchanged.	Normal.
1924. Jan. 10	*Crescents* swollen, *bands* present, occasional *large globules* (peripheral flesh).	Not prominent, distinct on boiling (H_2O).	*Papillae* not prominent.	Swollen pectic substance at the air-space angles.	Normal.
Feb. 8	*Crescents* altered, *bands* prominent, *large globules* present (peripheral flesh).	Slight development.	*Papillae* and *small globules* moderately prominent.	Swollen pectic substance at the air-space angles.	Occasional *bottle-shaped cells.*
Mar. 6	The changes have extended to the central regions of the flesh.	Prominent.	Very *small globules* present.	Cells show signs of separation.	Occasional *bottle-shaped cells.*
Mar. 15	Slight extension of changes.	Prominent.	*Small globules* prominent and numerous.	No further change.	Occasional *bottle-shaped cells.*
Apr. 2	Slight extension of changes.	Altered.	No further change.	No further change.	Occasional *bottle-shaped cells.*
May 15	Remains of *crescents* and *bands*, *large globules* numerous.	Much altered or absent.	No further change.	Pectic substances less prominent.	Cell-walls not well stained.
Aug. 6	Remains of *crescents* and *bands* and *large globules.*	Traces only.	In stages of disappearance.	Cells separating.	*Bottle-shaped cells* common, cell-walls show general lack of pectic substance.
1923. July 3	Remains of *crescents*, *bands*, and *large globules.*	Much altered or absent.	In stages of disappearance.	Cells separating, with strands of pectic substance between cells.	*Bottle-shaped cells* prevalent and cell-walls stain feebly and in patches (irregular areas).

accelerated, resulting in an accumulation of soluble pectin in the tissues. As the fruit becomes over-ripe the pectin breaks down further into soluble decomposition products and a decrease is detected then in the middle lamella pectic substances as they also pass into solution. This process of degradation continues gradually, a marked decrease in total pectic constituents being

observed during the last stages of senescence, and the pectin and middle lamella pectic substances disappear completely in extreme cases (see Text-fig. 2).

It may be concluded, therefore, from the investigations on both the chemical and microscopical sides, that the series of progressive changes in the pectic compounds which are distributed in the cell framework are intimately associated with the gradual metabolic drift in the apple from maturity to the final stages of senescence. It may be stated, in fact, with a considerable degree of assurance, that certain pectic changes are characteristic of the particular stage of maturity or senescence which a sample of fruit under examination has reached, and that the converse case may be usefully applied to the commercial aspect of the problem, namely, that the stage of development or maturity of a given sample of apples can be gauged approximately by either a microscopical or a chemical examination of the tissues.

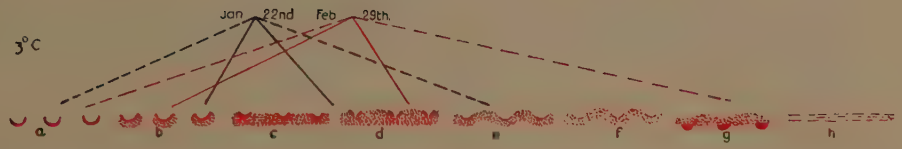
At the present stage of this work it is hardly possible to form a satisfactory conception of the mechanisms promoting pectic disturbances. Judging from the results obtained by the use of reagents on sections of tissue (see Section VI), the pectic decompositions appear to be a series of hydrolytic changes. The possibility of enzyme activity in the regulation of such changes is, of course, obvious, any such activity being regulated by modifications in the cell contents.

(c) *Effect on Changes in Bramley's Seedling Apples of Storage at Various Temperatures.*

No acceleration of the pectic changes was observed as a result of storing at a slightly higher temperature, i.e. 3° C. (Pl. XIII, Figs. 20–3), but a considerable rise in temperature, for instance to 15°, or storage under ordinary laboratory conditions, resulted in acceleration of the pectic changes (Pl. XIII, Figs. 18, 19). Thus the degree of pectic change reached by apples stored at 1° C. in March was comparable with that reached about three months earlier by apples stored under laboratory conditions. In Text-fig. 4 the differences in behaviour of the pectic substances at 3° C. and laboratory temperature is presented diagrammatically. These observations are in agreement with those made as the result of chemical estimations of the pectic changes in similar apples held at laboratory temperatures and at 1° C. respectively (10, 12).

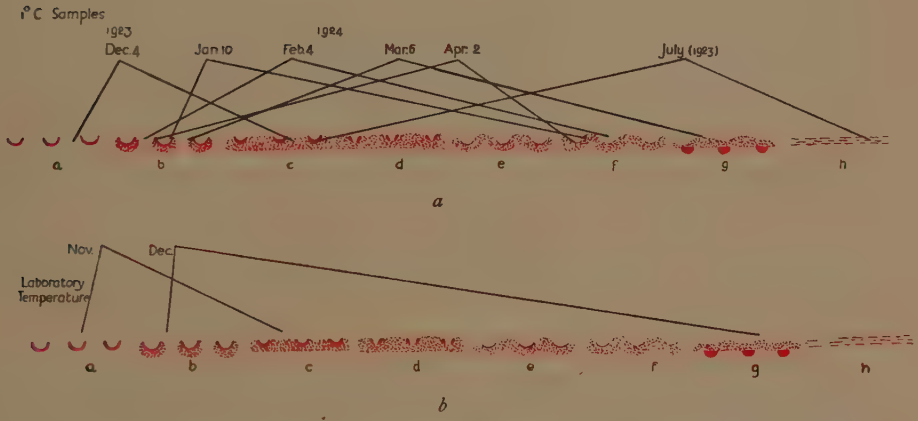
(d) *Variation in Changes in Bramley's Seedling Apples obtained from Various Localities.*

It was observed that the same variety of apple obtained from different localities and examined at the same time (November, 1923) exhibited varying degrees of pectic change. Thus, apples from Burwell (fen-land) and

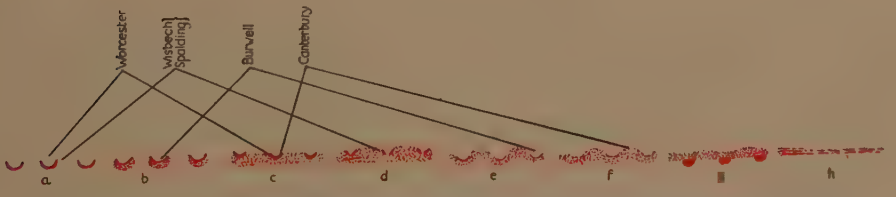


TEXT-FIG. 3. Variation in degree of change of crescents occurring among the individuals in samples of Bramley's Seedling apples stored at 1° C., and examined at different dates, i. e. January 22 and February 29.

Jan. 22 ——— minimum degree of variation in sample.
" – – – – maximum " " " " "
Feb. 29 ——— minimum " " " " "
" – – – – maximum " " " " "



TEXT-FIG. 4 *a and b*. Variation in the degree of change of crescents exhibited by different samples of Bramley's Seedling apples during senescence. 4 *a*. 1° C. 4 *b*. Laboratory temperature.



TEXT-FIG. 5. Variation in the degree of change in the crescents exhibited by samples of Bramley's Seedling apples from different localities examined at the same time.

Canterbury (gravel) showed more pronounced changes than those exhibited by apples from Spalding (silt), Wisbech (fen-land), and Worcester (Old Red Sandstone). Text-fig. 5 illustrates the variation in the degree of change exhibited by the crescents (as representative of the general trend of pectic changes) in apples obtained from five localities and examined at the same time.

(e) *Variation in Changes due to Variety of Apple.*

Generally speaking, the various phenomena exhibited by pectic substances in different varieties of apple do not differ markedly from those recorded for Bramley's Seedling. Differences in detail are found, for example, in size and number of the crescents, size and degree of distinctness of the discs, degree of prominence of the papillae, globules, &c. But in all the varieties examined the same type of change was observed, e.g. crescent alteration, development of small globules during late senescence, development of bottle-shaped cells, and progressive solution of the middle lamella pectic substances.

Differences in the rate of change were observed for different varieties which appear to be related to the maturity and keeping properties of the apple. Thus in the case of a rapidly maturing variety which is in season for only a short period, the sequence of changes is observed to take place much more rapidly than with Bramley's Seedling, a variety recognized as having good 'keeping' properties.

Among the varieties specially examined and compared were Bramley's Seedling, Bismarck (Pl. XIV, Fig. 29), Cox's Orange Pippin, Lane's Prince Albert, Newton Wonder, Allington Pippin, and Worcester Pearmain. Several of the more important varieties imported from the Dominions were examined also.

(f) *Pectic Changes in the Pear* (Pl. XIV, Figs. 47–51).

Preliminary observations made on the pectic substances present in pear tissues, during the period of storage at low temperatures, from the autumn until March, show that they differ in several respects from those of the apple, both in the forms which they assume and their behaviour during senescence. Thus, during the period of development the crescents which are characteristic of the apple are less frequently observed, and when present are much less conspicuous. For this reason the transformations which these structures undergo during senescence in the apple were not apparent. The development of numerous small globules in the free walls has not been observed. As with the apple, the cell-walls are not uniformly stained, but this feature is sometimes less prominent in the pear. On the other hand, the discs are numerous, but small. Pears in various stages of decay were

examined in March. The chief features observed were (see Pl. XIV, Figs. 47–51):

(1) Discs swollen and globular in form; (2) surface of the free walls, when stained with ruthenium red, exhibited a granular appearance; (3) the various manifestations of pectic change associated with the final dissociation of apple tissues were not observed, namely, the occurrence of irregular patches of pectic materials in the cell-walls, the extensive development of globules, and the presence of masses and strands of pectic materials between the cells; (4) bottle-shaped cells were prevalent.

In the case of apples in the condition of natural decay, the tissues readily dissociate when placed in water, or do so when gentle pressure is applied. This is not the case, however, with pear tissue; indeed, the cells often remain attached after the application of considerable pressure. This persistence in the cohesion of the cells appears, however, to be due to the presence of groups of stone-cells, which resist mechanical pressure and prevent its application to the parenchymatous cells, rather than to the presence of pectic substances acting as cell cement. This view is supported by the fact that if the stone-cells are first removed it is then possible to bring about the separation of the parenchymatous cells. Hence, although the microscopical examination of the pear reveals very few of the pectic phenomena common in the apple, the net result is the same in both fruits, involving more or less complete dissolution of the pectic framework accompanied by separation of the cells and plasmolysis of the cell contents. Since the processes of development, maturation, and senescence as a whole occupy a markedly shorter period of time in the pear as compared with the apple, it may be suggested that the lack of pectic structures is due to the temporary nature of their existence in a fruit which is undergoing more rapid metabolic changes.

The results obtained in this case of the pear[1] by adopting standard methods for extracting and estimating the various pectic constituents suggests that these constituents do not differ essentially, either in actual percentage content or in chemical constitution, from those present in apple tissues. This conclusion is borne out by the authors as a consequence of the results obtained by microchemical examination of pear tissue. Detailed comparisons of these fruits show that an important difference is a much lower acid content in pears as compared with most apples (Conference pear, 0·092 grm. malic acid per 100 grm. fresh weight; Bramley's Seedling 1·2 grm. malic acid per 100 grm. fresh weight of apple tissue).[2] It may be suggested, therefore, that acidity is the controlling factor in pectic metamorphoses, namely, in cell-wall degeneration, and that a low acid content involves a lessening of the protoplasmic control of the enzymes producing pectic

[1] Chemical investigations have been carried out by Miss A. M. Emmett, and the detailed results will shortly be published.

[2] See note, p. 213.

decomposition. It is interesting to note in this connexion that certain varieties of apples which have a low acidity (Cox's Orange Pippin, 0·666 grm. malic acid per 100 grm. of fresh weight, Beauty of Bath, 0·701 grm., and Worcester Pearmain, 0·191 grm.)[1] were proved to be those which very rapidly exhibit the tissue softening ('mealiness') which accompanies pectic decomposition.

VI. Effect of Hydrolytic Reagents on the Pectic Constituents of Apple Tissues (Pl. XIV, Figs. 26–8).

All the pectic transformations associated with the various phases of ripening and normal senescence of the apple fruit have been induced by treating sections with the appropriate solvents, viz. water, hydrochloric acid, potassium or sodium hydroxide, and ammonium oxalate, by carefully adjusting the temperature conditions and concentration of the reagents. Owing to the variation in the degree of pectic change occurring in individual apples, each experiment made in this connexion was repeated again and again until it appeared certain that the recorded changes were caused by the reagent employed. Two methods were adopted: (1) tangential sections were taken (using a radial cylinder of tissue) at a measured distance from the core, some of which were used for experimental purposes and the remainder kept as controls: (2) a particular section was chosen, and after recording the degree of change exhibited by the pectic structures, the section was then treated with the reagents and a second record made. On several occasions the same section was treated repeatedly either with the same or different reagents. This method was followed in all cases where inconclusive results had been obtained by the first method.

Sections soaked in water for short intervals of time (24 hours) gave variable results, but after more prolonged treatment, pronounced changes were evident, the crescents and discs had undergone alteration, and large globules were present. The changes observed in soaked sections were probably partly an effect of the lesions made in preparing the sections (see p. 223).

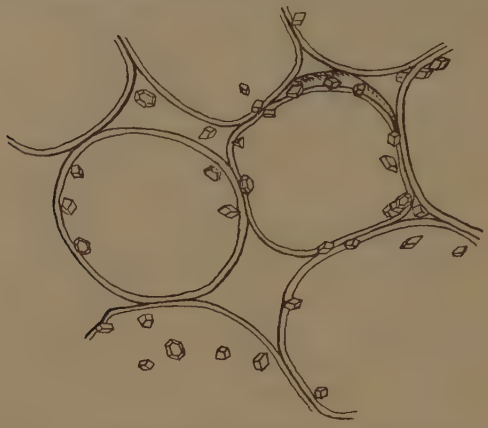
Boiling in water for periods of from 5 to 10 minutes induced considerable alteration in the pectic substances. In certain cases, by boiling sections in water and cooling rapidly, crystals were observed which were deposited round the cells following the lines of the middle lamella (Text-fig. 6). Further details are given in the accompanying table (Table III) of results from a representative series of experiments.

In Table III the dates of each experiment are given, since it is important to record the initial degree of development of the fruit before the experiment is begun, in order to arrive at a definite conception of the actual degree of alteration induced.

The results obtained from macrochemical investigations offer a ready

[1] These acid values were kindly furnished by Mr. D. I. Evans.

interpretation of the above phenomena (see Section II). Provided the sections are in a sufficiently immature condition, the treatment with boiling water



TEXT-FIG. 6. Crystalline deposit obtained by boiling sections in water and cooling rapidly.

TABLE III.

Illustrating the Effect of Boiling Water on the Pectic Substances present in the Tissues of Bramley's Seedling Apples.

Origin of the Apples and Storage Temperature.	*Date.*	*Condition of Pectic Substances before Treatment.*	*Treatment.*	*Condition after Treatment.*
Spalding (Silt), Lab. temp.	21. 11. 23	Crescents not observed.	Boiled 5 min. Reboiled 10 min.	Crescents numerous. Crescents less numerous and showing signs of change.
Burwell (Fen), Lab. temp.	23. 11. 23	Distinct crescents rare, bands feebly developed.	Boiled 5 min.	Few crescents, bands prominent.
Burwell (Fen), 1° C.	22. 1. 24	Crescents moderately abundant, some altered; bands narrow, discs distinct.	Soaked 24 hours, then boiled 5 min.	Crescents scarce, bands prominent, discs scarce.
Burwell (Fen), 3° C.	1. 3. 24	Crescents numerous, some showing signs of alteration, bands feebly developed, no large globules.	Boiled 5 min.	Crescents altered, large globules observed.
Worcester (Old Red Sandstone), 3° C.	10. 3. 24	Crescents numerous, some showing signs of alteration, no large globules.	Boiled 5 min.	Prominent bands, large globules observed.
Burwell (Fen), 1° C.	6. 3. 24	Crescents altered, bands prominent, discs indistinct, large globules numerous.	Soaked 18 hours, then boiled 5 min.	Large globules not observed.

may be supposed to hasten the production of pectin from the pectose in the tissues, and soluble pectic substances from the middle lamella complex, producing the usual transformations of the pectic structures. Treatment of

sections in a more advanced state of maturity produces the phenomena which are usually associated with the final stages of senescence, namely, the various substances are dispersed, their place being taken by large globules or diffuse stainable masses which after prolonged boiling completely dissolve and disappear.

TABLE IV.

Illustrating the Effect of HCl, KOH, and Ammonium Oxalate on the Pectic Substances present in the Tissues of Bramley's Seedling.

Condition of the Pectic Substances.

Before Treatment.	*Treatment.*	*After Treatment.*
(1) Crescents not observed. Bands not prominent. Discs numerous. Large globules absent. Cell-walls well stained (21. 11. 23).	(i) Soaked M/10 HCl 12 hrs.	(i) Little change.
	(ii) Boiled M/10 HCl 10 min.	(ii) Discs present. Cell-walls feebly stained. Tissue disintegrating.
	(iii) Boiled M/10 HCl, then soaked M/10 KOH 24 hrs.	(iii) General loss of pectic substance. Discs very indistinct. Cell-walls stain in patches.
(2) Distinct crescents few. Bands feebly developed. Discs numerous, indistinct. Cell-walls well stained (23. 11. 23).	(i) Soaked 2N HCl 12–72 hrs.	(i) Remains of crescents. Bands more distinct. Cell-walls well stained.
	(ii) Soaked 2N HCl 12 hrs., then soaked NaOH 12 hrs.	(ii) Outlines of crescents. Cell-walls stain in patches.
(3) Crescents moderately abundant and distinct. Bands present. Discs numerous and distinct. Cell-walls well stained (26. 11. 23).	(i) Boiled KOH.	(i) Crescents and discs absent. Cell-walls feebly stained. Tissue disintegrating.
(4) Apple exhibits advanced pectic changes (3. 7. 24).	Soaked 5 % HCl 3 days, then boiled 5 % KOH.	Pectic structures absent from cell-walls; lumps of pectic material scattered about the section.
(5) Crescents and altered crescents moderately abundant. Bands present. Large globules absent. Discs numerous, somewhat altered. Small globules present (7. 3. 24).	(i) Soaked H_2O (5 days).	(i) All pectic substances changed, much diffuse pectic substance present, and numerous large globules.
	(ii) Soaked ammonium oxalate 5 days.	(ii) Little pectic substance left, no large globules.
	(iii) Boiled ammonium oxalate.	(iii) Pectic substances absent; section disintegrating.

The effect of the water therefore appears to be a hydrolytic decomposition of the pectic substances already existing in a labile condition, followed by solution of the decomposition products.

Soaking in cold dilute HCl (M/100) failed to produce any marked pectic changes; the changes were more marked when the concentration was

considerably increased, but even then the cell-wall stained strongly with ruthenium red. Repeated boiling in dilute HCl (M/100) did not materially affect the pectic substances. More pronounced effects were obtained on increasing the concentration ; nevertheless, when the operation of boiling was continued until the section disintegrated, pectic substance was still present in the cell-wall, and globules, when present, were strongly stained with ruthenium red. Treatment of sections with cold dilute acid, followed by immersion in cold dilute alkali for sufficiently prolonged periods, produced marked changes (Pl. XIV, Figs. 26 and 27). Compare also Pl. XIV, Fig. 29. Boiling in HCl, followed by KOH or NaOH, caused disintegration of the sections and an almost complete disappearance of all the pectic constituents of the cells (Pl. XIV, Fig. 28). Boiling ammonium oxalate also brings about the disintegration of all the pectic substances. Some typical examples of the experiments made with HCl, KOH, and ammonium oxalate are given in Table IV.

The following interpretation is given in explanation of the above observations: All the pectic substances (pectose, pectin, pectic acid, &c.) are practically insoluble in cold dilute hydrochloric acid, hence no pectic changes are apparent. Boiling with dilute HCl (M/100) causes hydrolysis of pectose with production of pectin, and probably a certain amount of pectin break-down products of acid nature will be formed from this pectin, but owing to the insolubility of pectic compounds in hydrochloric acid, they are not removed by this treatment from the tissues, which continue to stain deeply with ruthenium red. The more pronounced effect on the pectic structures produced by increasing the concentration of the hydrochloric acid is attributable to its decomposing action with production of derivatives of non-pectic character. Subsequent boiling with caustic alkalis, however, causes disintegration of the tissues and entire absence of staining, since both pectin and pectic acid and the middle lamella pectic constituents are soluble in caustic alkalis, after previous treatment with hydrochloric acid. Boiling ammonium oxalate has much the same effect as hydrochloric acid followed by caustic alkalis, being a very efficient solvent for all the pectic constituents of the tissues.

VII. Observations on the Pectic Constituents of Plant Tissues other than the Apple.

The tissues of a limited number of plants representative of various types of plant structures have been examined, with a view to comparing the distribution and appearance of their pectic components with those found in apple tissue.

It was found that important differences occurred in the majority of the specimens examined, the structures on the whole being dissimilar to those

found in the apple. For instance, in the potato both crescents and discs were located in the cell-walls, but these were very minute and reacted feebly with ruthenium red. On the other hand, the middle lamella was distinctly outlined and the cell-walls did not exhibit the unequal staining so characteristic of those of the apple. These features in the potato are possibly related to the fact that the tissue of the potato is compact and the intercellular spaces are relatively small. In certain swollen root-structures such as the turnip and carrot, in the petiole of rhubarb, and in certain fruits, such as the orange, lemon, plum, gooseberry, and red currant, an abundance of pectic material which stained deeply with ruthenium red was observed in the cell-wall and middle lamella.

It was observed also that the development of the gooseberry fruit, which was followed from the time of fruit setting to ripening, was not attended by any special modifications of the pectic framework, that is to say, no crescents, discs, nor globules were discernible.

The presence of pectic compounds was confirmed in certain specialized tissue structures; for example, in the collenchyma of rhubarb, the epidermal and hypodermal cells and the cells of the parchment layer bounding the ovarian cavity in the black currant, the larger cells of gooseberry, and the small cells of the rind of the orange, lemon, and grape-fruit.

The older phloem elements in various plants often stain intensely with ruthenium red.

A special development of pectic substance is often found to be associated with the comparatively large pits which occur in the thick-walled cells, and notably in the irregular cells of the ground tissue of the rind of orange, lemon, and grape-fruit. When treated with ruthenium red and viewed from certain directions, the pits show up as structures of crescentic outline which recall the crescents observed in the apple fruit.

Mangin (38–45) showed that the formation of intercellular spaces in plant tissues was associated with various modifications in the pectic components of the cell framework, which he describes as 'boutons', 'bâtonnets', 'cadres d'union', &c. In the case of the melon and Narcissus it has been found that such modifications as Mangin describes do actually occur. With the melon various stages in the process of cell separation were observed. In the early stages the cells are held together by short cylindrical connexions which consist of intensely staining pectic substance ('cadres d'union'), and as development proceeds, these connecting strands become constricted and are finally ruptured as the tissue attains its maximum expansion. In consequence of this rupture large globules of pectic substance remain on the opposed walls of the separated cells ('boutons', 'bâtonnets').

VIII. Pectic Changes associated with Abnormal Conditions in the Apple.

The work on normal apples has been extended to an examination of pectic changes in apples in abnormal states, namely, apples affected by functional and fungal diseases and apples subjected to artificially induced conditions, such as exposure to gases, chloroform, &c.

(a) *Functional Diseases.*

Considerable confusion exists at the present time as to the classification of the functional diseases of the apple, especially those which make their appearance in apples held under low temperature or gas storage conditions. In all countries where the conservation of apples is carried out on a large scale under these conditions it is not an uncommon experience to find that a certain proportion decay prematurely. The tissues of the apples become brown, and in some cases tissue-softening ensues. The symptoms of decay may be confined to the general cortex, or they may extend from the cortex to the skin, in which case disease is evident at the exterior of the apple. The actual macroscopical symptoms are very variable: in some cases the brown discoloration is fairly generally distributed, in others it is confined to the central region of the apple, and in still other cases the tissues bordering the vascular bundles are prominently discoloured. The brown areas are sharply defined in some cases, whilst in others they merge gradually into the uncoloured tissues. Cavities may or may not be present in the brown flesh, and the formation of cavities may precede the development of brown areas. Different varieties of apple are not affected alike when held under similar storage conditions. Again, the same variety of apple may exhibit different symptoms in different years although the storage conditions have remained practically unaltered.

Reference to the literature relating to the various functional troubles shows that the classification attempted has been based almost entirely on macroscopical characters, microscopical characters being rarely taken into account. It is thus practically impossible to compare accurately one form of functional disease with another. Again, the macroscopical descriptions are often incomplete; thus in describing a particular functional disease it is often not stated whether the tissues are soft or firm. Since a condition of softening would be brought about by the solution of the middle lamella consequent upon pectic disturbance, the presence or absence of softening would afford an important clue as to whether the chemical reactions taking place in the different categories of functional disease were similar or otherwise.

For convenience, the classification adopted by Kidd and West (35) has

been followed in describing the pectic changes observed in the various forms of functional disease studied by the writers.

The following observations should be regarded strictly as of a preliminary nature. The whole question of the symptoms characterizing the functional diseases of the apple needs a much fuller investigation.

1. *Internal break-down* (Pl. XIV, Figs. 30–2). This name is used by Kidd and West to designate certain functional troubles which occur when apples are stored in ventilated chambers under low temperature conditions. Under this name are classified forms of disease where tissue-softening occurs, and forms where the tissues remain firm.

The soft type of break-down is often prevalent among apples stored at 1°–3° C. In the Bramley's Seedling variety it is usually evident at the exterior of the apple; the skin becomes discoloured and the sub-cuticular tissue yields readily to pressure. The first signs of the disorder are usually to be detected in the peripheral region of the cortex, whence the disease spreads until the whole apple is rotten. The condition is illustrated in Text-fig. 7. A microscopical examination of an apple affected with this soft break-down at the mid-period of storage yields the following results. In the brown region of the cortex the pectic substances of the cell-walls exhibit change and have completely disappeared from the oldest brown cells. The middle lamella is dissolved and stringy masses of pectic material occur between the cells (Pl. XIV, Fig. 32). The tissue at the margin of the brown area exhibits intermediate pectic changes. The crescents and discs usually show advanced stages of decomposition. The cell-wall pectic substances are unevenly distributed and globules of various kinds are prevalent. As the sound tissue is approached, the pectic disturbances become less and less evident, and in the healthy tissue no abnormal changes are found.

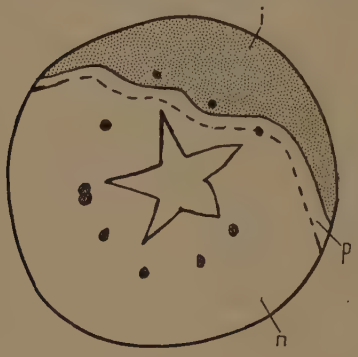
This series of pectic changes is entirely analogous to those changes prevailing during normal senescence, differing mainly in the rapidity with which the changes are effected, and also in the completeness of the pectic decomposition attained, which in normal apples is observed only in the most extreme stages of senescence, such as are rarely found owing to the prevalence of fungal invasion.

The later the onset of internal break-down, the less is found to be the contrast between the condition of the pectic substances in the sound and diseased portions of the apple, because the normal changes are trending in the same direction as those which are taking place prematurely in internal break-down.

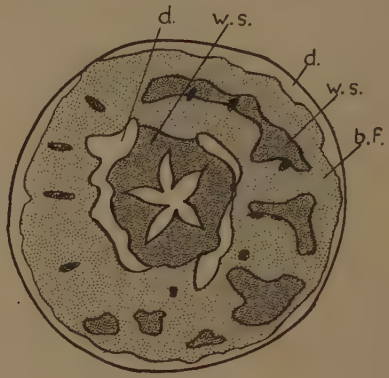
It is not possible to make an exact differentiation between apples affected with internal break-down and apples in a state of natural decay. Generally speaking, in the former case the trouble spreads inwards from sub-epidermal and local areas of the cortex and the tissues are brown, soft,

and watery; in the latter case the cortex is more or less uniformly 'mealy', and the tissues are not always discoloured when the cortex is in a state of 'mealiness'.

It was observed that the pectic framework is not necessarily uniformly affected by the processes which bring about the hydrolysis of the pectic substances of which it is composed. Thus in some cases the crescents and



TEXT-FIG. 7.



TEXT-FIG. 8.

TEXT-FIG. 7. Transverse section of an apple affected with internal break-down (median plane), showing diseased areas. *n*, normal tissue; *p*, zone where partial pectic changes occur; *i*, brown soft tissue where advanced and final changes are found.

TEXT-FIG. 8. Transverse section of a Jonathan apple (U.S.A.) affected with internal browning (December 9, 1925), median plane. *w.s.*, dark water-soaked tissue; *b.f.*, brown firm tissue; *d.*, slightly discoloured tissues. Disease not evident at exterior of apple.

discs have undergone solution before the middle lamella is decomposed; in others the reverse has happened, when unaltered crescents and discs may be observed associated with the walls of completely separated cells.

On some occasions when apparently healthy apples were examined it was found that certain areas in the peripheral cortex exhibited a more advanced state of pectic change than was evident in the surrounding tissues. These areas may be interpreted as centres of incipient internal break-down, and it may be possible therefore to predict the imminence of internal break-down in stored apples by examining a sample microscopically.

The firm type of internal break-down was found in Bramley's Seedling apples kept in cold storage at $-1°$ C. at the Low Temperature Station, Cambridge. These apples, for which the writers are indebted to Dr. West, were examined in April, 1924. The apples were green to greenish yellow in colour and showed externally no signs of disease. On cutting them open the cortex was found to be pale brown in colour. The whole of the cortex was affected, with the exception of a narrow zone of cells beneath the skin. A microscopical examination showed that the relatively slight browning was due to the fact that uncoloured living and brown dead cells occurred

intimately intermingled throughout the tissues. A comparison with normal apples stored at 1° C. showed that comparatively little abnormal pectic change had taken place. The middle lamella was intact, which accounted for the firmness of the tissues. Bottle-shaped cells, which are usually a striking feature in tissues affected with the soft type of internal break-down, were almost absent. The condition of the dead cells somewhat resembled that found in apples which have been exposed to chloroform vapour for a short period, in which a more prominent development of bands is the only sign of any abnormal pectic change. The symptoms described above, namely, the firmness of the tissues, the peculiar distribution of the dead and living cells, and the unaltered cell-walls, correspond very closely with those described by Winkler (68) as present in apples affected with 'internal browning', a functional disease sometimes prevalent among apples when stored under low temperature conditions (− 1° C. to + 5° C.) in the United States.

In the autumn of 1925 the authors obtained some specimens of Jonathan apples which were traced to a shipment from Washington. These apples were firm, green, and entirely free from external symptoms of disease, but internally they were diseased to such an extent that extremely little sound tissue remained. Text-fig. 8 shows the general features which were observed when one of these apples was cut in half. The general mass of the tissues (*b.f.*) was of a medium brown colour. The brown tissue extended to within a few millimetres of the skin, leaving a narrow, slightly discoloured zone (*d.*). Water-soaked areas (*w.s.*) were present in the tissues, the area in the centre was light brown, the remainder very dark brown in colour. These general symptoms agree with those described by Winkler (68) as characteristic of the late stages of 'internal browning'. The condition of the tissues in this advanced state of disease was still unassociated with any extensive disturbance of the normal sequence of pectic changes. Typical cells from the diseased tissues of the apple illustrated in Text-fig. 8 are represented in Pl. XIV, Figs. 33 and 34.

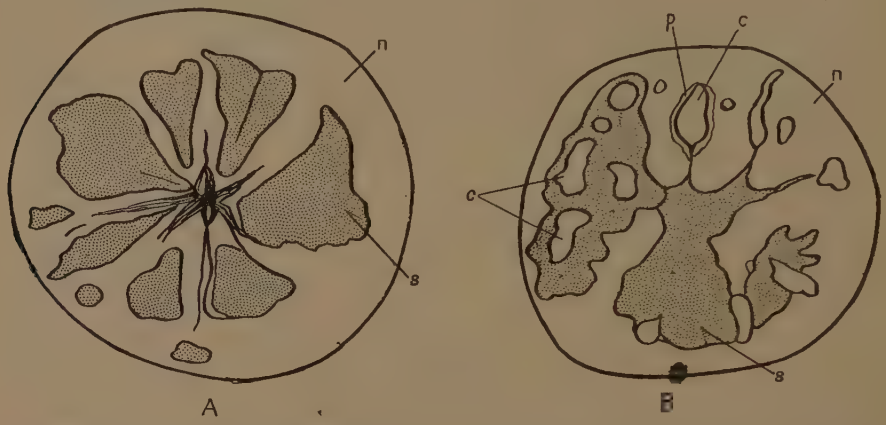
2. *Scald* (see Brooks and Cooley (7)). Under this category, Kidd and West include (α) browning of the superficial cells of the apple unaccompanied by softening or browning of the flesh, and (β) the various 'spotting' diseases of apples which are not due to fungi.

The authors' observations have been confined to cases of typical scald (α). The troubles included under (β) also include spotting due to the presence of sub-cuticular, bitter-pit areas (28–9) which should be classified under bitter pit.

In the cases of scald examined, the skin of the apple was locally discoloured and sometimes slightly indented. This appearance is due to the death and browning of epidermal and of one or more layers of sub-epidermal cells. Very little difference was detected between the pectic constituents of

the dead and those of similarly situated normal cells. The parenchymatous cells adjacent to the affected tissue, however, exhibited partial pectic changes—altered crescents, globule formation, &c. The middle lamella was not dissolved.

Apples affected with scald frequently develop tissue-softening when held under cold storage conditions after scald has appeared. The softening commences in the tissue underlying the layer of dead cells of scald origin and spreads internally. The microscopical effects produced are identical with those associated with internal break-down.



TEXT-FIG. 9. Transverse sections (median plane) of a Newtown apple affected with brown heart under gas storage conditions (May 9, 1924). (A) Section of an apple taken 2·5 cm. from the stalk end. (B) Section of an apple taken 1·5 cm. from the flower end of the same apple. *c*, cavities; *s*, soft brown tissues; *n*, normal tissue; *p*, zone of partial pectic disturbance.

3. *Brown Heart.* Under this heading, Kidd and West (36) describe a functional disease which is encountered among apples held in gas storage. The apples are affected with tissue browning which is usually not evident until the apples are cut open (Text-fig. 9, A and B). Large cavities [1] (Text-fig. 9, B) in the flesh are of common occurrence, attributed by Kidd and West to the 'drying out' of the decaying tissues. The following microscopical details were observed in a small sample of apples for which the authors are indebted to Dr. Kidd. Both softened tissue regions and cavities were present in the specimens received. The softened tissue exhibited the pectic disturbances characteristic of apples affected with internal break-down —hydration and disappearance of the crescents, extreme development of globules of pectic substance, and dissociation of the cells, with the usual formation of bottle-shaped cells. Each cavity was bordered by a narrow layer of crushed cells, the walls of which were impregnated with pectic sub-

[1] The formation of cavities or hollows in the flesh of apples is of course not confined to those affected with 'brown heart'. Cavities may be induced by submitting apples to various experimental conditions.

stance. The living cells surrounding the crushed layer exhibited partial pectic disturbance as seen in the cells bounding a bitter-pit area.

4. *Bitter Pit.* Pectic changes of various kinds occur in tissues affected with bitter pit. The cells in a typical bitter-pit area are dead and shrivelled. Occasionally a wound cambium is present in the neighbourhood of the dead cells, the new cells formed often projecting in a filamentous manner into the air-spaces. The walls of the dead cells stain intensely with ruthenium red, but the discs, crescents, and globules have disappeared. The cells do not separate, the rents in the tissue appear to be produced mechanically through cell contraction. Partial pectic changes are found in the narrow zone of cells surrounding the diseased area.

5. *Water Core* (see J. B. S. Norton, 'Phytopathology I', pp. 126–8, 1911; P. J. O'Gara, 'Phytopathology III', pp. 121–8, 1913; F. D. Heald (27), and others). The tissues in the water-soaked region of apples affected with water core were examined with a view to ascertaining to what extent, if at all, the infiltration of the intercellular spaces by water had brought about changes in the pectic constituents of the cells. No changes which could be associated with the waterlogged condition were detected.

It follows from the foregoing account that considerable differences are observed in the pectic disturbances obtaining in the different functional diseases. These differences may be attributed to variations in the factors promoting pectic decomposition, peculiar to the disease in question, since it is probable that the above-described diseases are due to abnormalities in the metabolic processes, thereby producing variability in the mechanisms capable of causing decomposition of the pectic constituents of the tissues. These abnormalities may be associated with the observed variations in the decomposition of the pectic substances, especially in view of the inherent differences which exist in their composition.

(b) *Artificially induced Conditions.*

Certain pectic changes are induced by tissue lesions, Pl. XIV, Figs. 39–41, provided that the material subjected to lesion is obtained from mature apples in which the pectic constituents are already existing in a condition of incipient decomposition.

The pectic disturbance is confined to a narrow zone of tissue surrounding the cut surface. The discs and crescents in this area show marked signs of alteration, and large globules are of common occurrence. The middle lamella remains unchanged.

Similar and more pronounced changes are produced by the action of gases. Whole apples (Bramley's Seedling) in which incisions had been made were submitted to the atmosphere of various gases in sealed jars, except in the case of ozone, when a special ozonizer was used. Precautions

were taken to ensure that the observed changes were actually produced *in addition* to those resulting from lesion.

In the case of oxygen and ozone the changes do not differ markedly from those due to tissue lesions, and are limited to a narrow zone of cells surrounding the cut surface. The discs and crescents are altered, prominent bands occur, and globules are prevalent. The middle lamella appears to be unaffected.

With nitrogen and carbon dioxide the pectic changes are similar to those obtaining in internal break-down (Pl. XIV, Figs. 35–8). The tissue becomes brown and softens, the cells tend to separate, and stringy masses of pectic material are deposited between the cells—globules are prevalent, the discs and crescents disappear, and finally there is considerable diminution of the pectic substances. Somewhat different symptoms were obtained when varieties other than Bramley's Seedling were used. When uncut apples of certain varieties were kept in an atmosphere of CO_2 for some time the skins became discoloured.

When mature apples are exposed to the vapour of chloroform or ether for about two hours, the tissues become brown owing to death of the cells, but there are no marked signs of pectic disturbances. The middle lamella is not affected.

The effect on the pectic substances of these various gases and of lesions is thus widely different. Oxygen and ozone appear to affect only the cells in the cut area in immediate contact with them, and the slight effect produced may be at any rate primarily due to lesion. Chloroform and ether appear to exert an inhibitory action on the mechanisms producing pectic decomposition, and death of the cells results from the treatment. Tissue lesion appears to have the sole effect of accelerating the pectic changes in the immediate area affected. This local effect may be attributed to the liberation of enzymes or cell contents capable of causing decomposition of the pectic substances. The effect of nitrogen and carbon dioxide in accelerating pectic changes is curious, and may possibly be due to the piling up of unoxidized products which directly or indirectly accelerate pectic decomposition.

(c) *Fungal Diseases* (29).

A preliminary study of the effect of fungal invasion upon the pectic constituents has been made. The fungi used included species, such as *Cytosporina ludibunda*, previously found by the authors to be capable of causing decomposition of pectin, together with *Pleospora pomorum* and a species of *Fusarium* which do not appreciably bring about such decomposition.

It was observed, in certain cases of fungal attack (Pl. XIV, Figs. 42–6), that the cells of the host tissue occupying a narrow zone immediately in

advance of the invading organism were dead as evidenced by their refractive index and plasmolysed contents. In tissues unaffected by *Fusarium* and *Pleospora pomorum*, the middle lamella appeared to be intact, and no marked disturbances of the other pectic constituents were manifest either in the zone of dead cells or in the cells in the region occupied by the invading hyphae. Tissues attacked by *Cytosporina ludibunda* showed pectic changes in the zone of dead cells in advance of the fungal hyphae (Pl. XIV, Fig. 42), as evidenced by the presence of crescents in various stages of alteration and by globules of pectic substance. More advanced changes were observed in the tissues actually invaded by the fungus (Pl. XIV, Figs. 43–6), and in some extreme cases an almost complete disappearance of the pectic constituents was observed.

In all the cases examined, partial or complete cell separation is invariably observed in the invaded tissues. This may be entirely or in part a mechanical effect caused by hyphal penetration between the cells. In the case of *Cytosporina*, however, the evidence suggests that cell separation is partly due to the solution of the middle lamella. The disintegrating action of certain bacteria on plant tissues is well known, and this action has been attributed by Winogradsky (64), Marshall Ward (61), de Bary (3), Brown (8), and others to the decomposition of the pectic constituents of the middle lamella by enzymes secreted by the bacteria themselves.

Brown (8) has shown that certain fungi, notably *Botrytis*, are able to excrete a toxic substance which kills the cells of the host. Since in all the cases cited above the host cells are killed in advance of the invading hyphae it is highly probable that the death of these cells is due to a fungal toxin. In those cases of fungal invasion which are not characterized by any marked change in the pectic constituents an effect analogous to that caused by anaesthetics is evident, and it may be assumed that cell-death and the absence of pronounced pectic changes are simultaneous effects produced by the same toxic agent. The case of *Cytosporina ludibunda* is essentially different in that this fungus is capable of causing the decomposition of pectic substances. Not only are pectic disturbances and loss of pectic substance evident in the invaded tissues, but the disturbances extend to the tissues in advance of the invading hyphae. Hence it is not unreasonable to suppose that these disturbances are due to the partial or complete utilization of the pectic substances by the fungus itself as a source of food.

IX. General Discussion.

It has been shown that the pectic substances are in a state of flux from the end of the flowering period onwards to the period of natural decay. From the setting of the flower to the maturity of the apple the tissues, including the intercellular system of the apple, are developing. The pectic

substances which are uniformly distributed in the middle lamella and cell-walls when the tissue is compact undergo rearrangement, with the result that the middle lamella occupies a smaller area relative to the size of the cell-walls, and a specialized pectic framework is elaborated which comprises pectic structures of definite shape—crescents, discs, globules, &c. This framework differs in structural detail from similar structures observed in many other plants (see Section VII).

During senescence the pectic components of the tissues undergo further changes. They appear to be in a more labile condition, and masses of semi-soluble pectic matter occur in the cell-walls. Globules are developed, comparable to those found by Mangin in other plant tissues, but not associated by him with a particular phase in development. Consequent upon these changes occurs a gradual lessening of the cohesion of the cells and a modification of the intercellular spaces. The changes proceed very slowly, and culminate in complete decomposition of the pectic compounds, resulting in their total disappearance as revealed by staining. At this stage the cells can be separated from one another by slight mechanical pressure owing to solution of the middle lamella. Plasmolysis of the cells is observed, and many of the cells assume a curious bottle-shaped form.

The study of the normal sequence of pectic changes is often obscured by the incidence of various functional diseases. For instance, internal break-down was prevalent to a greater or less extent in all the years during which this investigation was proceeding. In some seasons when internal break-down was less prevalent a certain percentage of the apples reached the condition of natural decay, whilst in other seasons internal break-down ensued at an early stage, so that the great majority of the apples were prematurely and abnormally broken down. Since the apples were obtained from the same orchard each year, and since approximately the same interval of time elapsed between gathering and placing in cold storage, these differences must be attributed to factors affecting the constitution of the apples before gathering. In this connexion it is interesting to note that Winkler (68) found that the development of functional disease in the Yellow Newton apple was influenced by climate, soil, and methods of orchard practice.

It has been found possible, by microchemical treatment, to imitate the changes which occur during senescence, and since such changes have been effected by the use of hydrolytic reagents (see Section VI), they are interpreted as the result of progressive decomposition of the pectic substances by a process analogous to hydrolysis.

It has also been shown that microscopical changes can be correlated with those observed by quantitative chemical methods which have been independently interpreted as due to the hydrolytic decomposition of the pectic substances (see Section V).

The exact nature of the pectic substances constituting the pectic framework during its development, and the series of changes leading to its decomposition, have already been discussed in some detail (see Section V, and also (12)). At least three forms of pectic substance have been detected—pectin and pectose in the cell-walls, and the pectic constituents of the middle lamella. It is probable that many forms of intermediate composition and varying solubilities occur between these three, and the evidence obtained from microscopical investigation also suggests that the pectic constituents of the cell-wall cannot be regarded as simply insoluble pectose which passes gradually into soluble pectin, but rather that transition compounds arise which may be generally referred to as pectinic acids (see (12)).

The following microscopical observations support this hypothesis: (1) When the tissues are treated with various reagents, the pectic substances are not uniformly acted upon; for instance, the crescents are more readily affected than the middle lamella. (2) The development of the crescents, discs, and globules is not attained simultaneously. (3) The transformations of the crescents and discs do not always take place concurrently with the solution of the middle lamella. (4) Tissues in a condition of 'internal break-down' may exhibit solution of the middle lamella before changes in the crescents and discs become apparent or vice versa (see p. 220).

The following attempt at the correlation of observed microscopical phenomena with the pectic metamorphoses as observed by chemical methods must be regarded as provisional, since it has not so far been found possible to identify with certainty the various structures observed microscopically with those pectic compounds ascertained by independent chemical methods to be present in the tissues.

The pectic framework as it exists in the undeveloped fruit may be conveniently dealt with under two headings: (1) The middle lamella pectic complex; (2) The cell-wall pectic complex.

1. The middle lamella pectic complex may be regarded as containing basal molecules of pectic acid, or salts of pectic acid, since throughout development until complete disintegration of the tissues is observed, it has been found possible to carry out an estimation of the middle lamella pectic constituents in terms of pectic acid. The initial stages of middle lamella decomposition may be conceived as involving the splitting off and solution of these groupings combined with the pectic acid molecule. These groupings, being no longer associated with pectic acid, have lost the typical pectic character of staining with ruthenium red, and the residual pectic acid will therefore tend to coalesce into masses of stainable material, and may be the cause of the appearance of the discs observed in the region of the middle lamella (Table V, 1, B). The crescents may similarly be interpreted as dense aggregations of this liberated pectic acid which gradually accumulate round a nucleus of some other cell constituent, possibly the protoplasmic cell con-

nexions. Pectic acid is insoluble in acid media, but slowly soluble in water, from which it follows that these water-insoluble discs and crescents may be expected to persist until the acidity of the cell sap diminishes as ripening proceeds. The observed facts show that changes in these pectic structures are very gradual and tend to become more marked as the fruit becomes less acid. The formation of bands (Table V, I, C) associated with the crescents may be attributed to the gradual imbibition of water by the pectic acid residues, ultimately producing the swollen masses which assume the form of large globules (Table V, I, G). Meanwhile decomposition of the pectic acid itself has probably set in, since in the final stages of senescence no pectic substances of middle lamella origin are apparent, and chemical estimations show a decrease in middle lamella pectic substances at this period. This chemical change is made apparent by the gradual decrease in the size of the globules, their corroded appearance, and their diminished capacity for retaining stain (Table V, I, H and J), until finally no traces of the original middle lamella structures remain (Table V, I, K). This interpretation is supported by a comparison of the changes in the various pectic structures as observed microscopically (see Table II), with the chemical changes in the middle lamella pectic complex shown by the graph in Text-fig. 2, *c*.

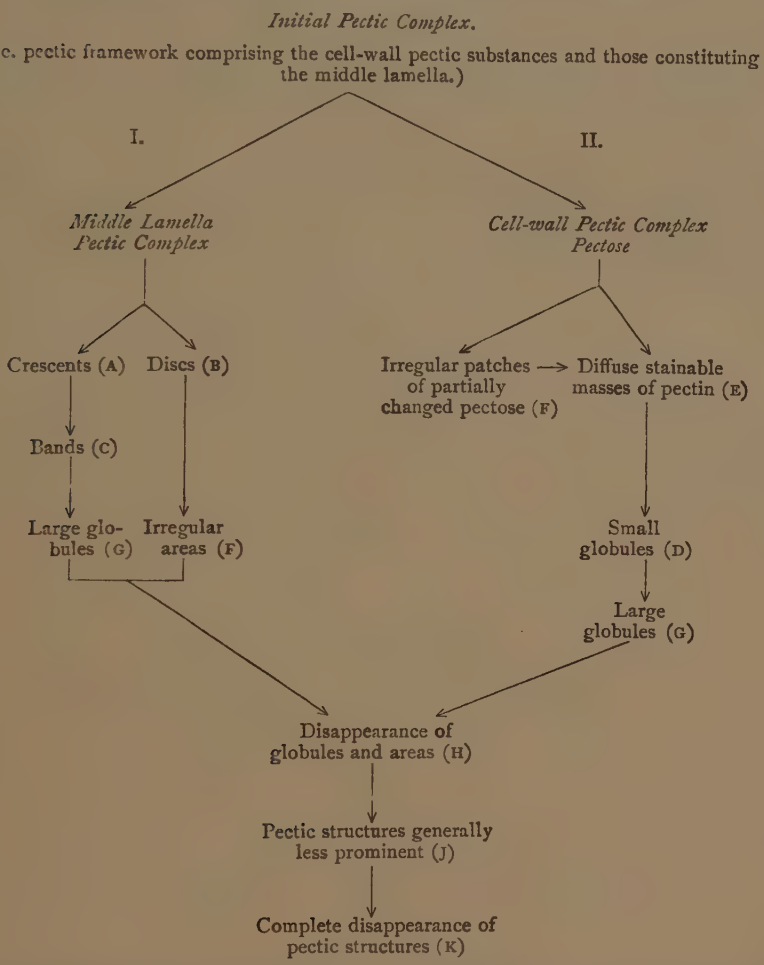
2. Meanwhile, in the case of the cell-wall complex, pectose is undergoing a similar series of degradations, but these are more difficult to follow, since the pectin produced by decomposition of pectose is readily soluble, and it is therefore difficult to ascertain its presence by microscopical methods. The appearance of irregular-shaped staining patches (on the free walls), which take the place of the uniformly staining walls of the undeveloped fruit (Table V, II, F), may be interpreted as residual masses of partially changed pectose, the pectin produced often being observed as diffuse stainable masses in the air-spaces or floating in the medium in which the section is examined (Table V, II, E). This was especially marked when sections were examined in alcohol in which pectin is insoluble. The appearance of small globules (Pl. XIII, Fig. 18) and later of larger globules (Table V, II, D and G; also Pl. XIII, Figs. 19 and 23), which are not readily soluble in water, may be attributed to the subsequent decomposition of pectin into the less soluble pectic acid, and the final stages of pectose decomposition will therefore present features entirely analogous to those exhibited during the disintegration of the middle lamella pectic complex (Table V, II, H, J, K.).

This interpretation of the microscopical phenomena associated with the disintegration of the pectic constituents of the cell-wall is in accordance with the estimated chemical changes, as will be seen by referring to Text-fig. 2.

From a consideration of all the results obtained in the course of these investigations, it is evident that the pectic changes induced in apple tissues

TABLE V.

Schematic Representation of the Origin and Nature of the Various Substances constituting the Pectic Complex.



Dissolution of the middle lamella pectic complex with production of difficultly soluble pectic acid (A), (B), gradual swelling of the pectic acid by water imbibition (C), (F), (G), followed by its solution and decomposition (H), (J) into products of non-pectic character (K).

Dissolution of the cell-wall pectic complex with successive production of soluble pectinic acids and pectin (E), leaving areas of modified pectose (F). Decomposition of the pectin (E) into pectic acid (D), (G) with the consequent production of the phenomena observed in (H), (J), (K).

by different agencies are of a somewhat varied character, although exhibiting a similar trend. All the cases of pectic changes may be grouped

together into three classes: (1) Pectic changes involving solution of the middle lamella and general pectic disturbances. These conditions are found in internal break-down (*soft* type), brown heart (regions where soft decay occurs), and apples kept in nitrogen or carbon dioxide. The phenomena observed in these cases are similar to those found in tissues in an advanced state of physiological break-down. (2) Cases where the middle lamella is not dissolved and only partial pectic changes occur. These conditions are associated with lesions, scald, bitter pit (in the cells surrounding the diseased areas), brown heart (in the cells surrounding the cavities), and apples submitted to the action of oxygen or ozone. The changes typical of this class are analogous to those produced in the early stages of senescence. (3) Cases where browning of the cortex is not associated with solution of the middle lamella, and no marked pectic changes occur. This condition is found in the *firm* type of internal break-down (internal browning), in tissues attacked by fungi in advance of the invading mycelium, and in apples exposed to the vapour of ether or chloroform. It is never associated with normal senescence.

The various pectic changes observed in apples, whether associated with the stages of normal senescence or connected with apples in abnormal states, are amenable to the general interpretation, that the pectic decompositions are brought about by enzyme activity under the control of the chemical conditions prevailing in the tissues. It is conceivable that the chemical composition of the unripe fruit exercises an inhibitory effect on the enzymes present, and the gradual changes taking place in the cell contents appear to have the subsidiary effect of stimulating enzyme activity; the pectic changes in consequence become increasingly prevalent, generally leading to the completely disintegrated condition typical of advanced senescence.

In the cases presented by apples in abnormal states, the instances where partial pectic changes occur, as with lesions, may be interpreted as the result of a partial or temporary lessening of protoplasmic control over certain enzymes capable of producing all the pectic changes except middle lamella solution. The phenomena produced in internal break-down (*soft* type), and as a result of exposure to carbon dioxide and nitrogen, may conceivably result from a more extensive and premature action of the factors controlling enzyme activity. In the case of apples submitted to the action of chloroform and ether (where death of the cells does not seem to involve any marked disturbance of the pectic compounds), the anaesthetics appear to have the double effect of killing the cells and of simultaneously arresting the pectic decompositions which normally accompany the death of the cells. The effect produced by anaesthetics supports the view advanced by Winkler to explain internal browning in the Yellow Newton apple. With Bramley's Seedling this form of pathological trouble occurred in apples stored at temperatures below 1° C., whereas the *soft* type of internal break-down occurred at 1° C. and higher temperatures. At

first sight this difference in response might be attributed to temperature. Winkler, however, observed internal browning at temperatures ranging from $-1°$ C. to $5°$ C.; hence the phenomenon is not solely related to temperature. Winkler attributes the condition of the tissues in internal browning to the toxic effect of certain organic compounds accumulated at low temperatures. The condition of the cells and the pectic constituents in chloroformed tissue and tissues affected by internal browning are analogous. Hence it is possible that different lethal agents, in one case of an anaesthetic, in the other of a toxin, are able to produce parallel effects. In cases of fungal invasion the same double effect is observed, viz. cell death and little pectic disturbance, and these facts admit of a similar general interpretation, the lethal agent being presumably in this case a toxin of fungal origin.

The disease known as bitter pit may be classed with the cases suggestive of toxic poisoning, the condition of the dead tissue being somewhat analogous to that found in chloroformed tissue, except that, through a process of 'drying out', the cells in the diseased area are ruptured.

Taking into account all the facts brought to light in the course of these investigations, the writers have so far found no ground for supposing that the dissolution of the pectic substances is a primary cause of the death of the tissues. On the contrary, the metabolic changes in the cell contents obtaining in the final stages of senescence appear to have the dual effect of inducing the complete decomposition and solution of the pectic substances and of causing plasmolysis and death of the cells.

X. Summary.

1. In this investigation the results obtained from a microscopical study of the distribution and developmental changes undergone by the various pectic structures in the tissues of the apple fruit are correlated with those accruing from a parallel and purely chemical study of the pectic materials present in apples of the same varieties, derived from the same sources and kept under the same experimental conditions. The results obtained in the two investigations conducted independently are in agreement.

2. The microscopical side of the work has been rendered possible owing to the extreme reliance which can be placed on ruthenium red as a reagent for detecting the presence of pectic substances in apple tissues not previously acted upon by fixatives or preservatives of any kind.

3. From a comparatively simple structure, as observed in the tissue at the time when the fruit is 'set', the pectic complex becomes elaborated during fruit development into a specialized and relatively stable framework, which is revealed as a stainable residue, and amenable to the action of pectic solvents after the cellulose constituents of the cell-wall have been removed by the action of Schweitzer's reagent. During the period of senescence

the pectic framework becomes less stable, and the various structures which constitute the framework may be differentiated from one another by the degree of resistance they offer to the action of pectic solvents. This instability synchronizes with the appearance of soluble pectic derivatives (pectinic acids and pectin) in the expressed juice of the apple, as determined by chemical methods. The quantity of pectin obtained by chemical extractions gradually increases during the period when the less stable constituents of the pectic framework exhibit the maximum degree of change as observed by microscopical methods. At the same time the greater resistance exhibited in time by the more stable constituents of the pectic framework towards solvents is in accordance with expectation, since after the extraction of the soluble pectic compounds less soluble pectic substances may be extracted from the residues by reagents of greater hydrolytic power. From their lack of synchronism, both in development and in the transformations they undergo during senescence, the various structures composing the pectic framework appear to differ among themselves constitutionally, thus supporting the view advanced from the chemical side of the complex nature of the pectic constituents of the cells.

4. All the changes in time exhibited by the less stable pectic structures under normal conditions can be imitated by treating sections of the tissues with hydrolytic reagents. Hence the normal transformations which these structural forms undergo are regarded as brought about by some process of hydrolysis. This view coincides with that already advanced in interpretation of the chemical results.

5. A brief description is given of the pectic framework in the pear fruit and the changes in time which this framework undergoes (Section V. (*f*)).

6. Certain structural forms assumed by the pectic compounds in the apple, e. g. globules, &c., appear to be of common occurrence in plant tissues; other structural forms, crescents, discs, &c., have not been observed in the tissues of the majority of the plant structures examined.

7. The methods adopted in this investigation have been applied to the study of the pectic changes which occur in apples in abnormal states, e. g. physiological and fungal diseases and artificially induced conditions, and certain results of a preliminary nature are given (Section VIII).

The authors are greatly indebted to Professor V. H. Blackman for his kind help and criticism.

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EXPLANATION OF PLATES XII–XIV.

Illustrating Drs. Carré and Horne's paper on an Investigation of the Behaviour of Pectic Materials in Apples and other Plant Tissues.

The authors are indebted to Miss M. Reeks, Technical Artist in the Imperial College, for the text-figures and plate drawings in this paper. Figs. 2–4 and 6–14 are purely diagrammatic; the remainder are semi-diagrammatic representations of drawings made with the aid of the camera lucida.

PLATE XII.

Fig. 1. Transverse section of young tissue (May 29, 1924) showing small intercellular spaces (*s.*) and free walls (*f.*).

Fig. 2. Portion of cell (July 2, 1924) showing the first appearance of discs (*d.*).

Fig. 3. Portion of cell (July 16, 1924) showing bands (*b.*) and discs (*d.*).

Fig. 4. Portion of cell (August, 1924) showing bands (*b.*), discs (*d.*), and crescents (*k.*) in early stages of formation.

Fig. 5. Cell from mature apple (December 4, 1923) showing walls of contact (*w.*), discs (*d.*), bands (*b.*), and crescents (*k.*). Also free walls (*f.*) and small globules (*g.*).

Figs. 6–14. *Series of diagrams illustrating the pectic changes which take place in the region of a wall of contact* (*w.*) *during the development and senescence of the apple.*

Fig. 6. Cell (September) showing fully developed discs (*d.*) and crescents (*k.*). Bands (*b.*) absent, free walls (*f.*).

Fig. 7. Senescent changes—crescents (*k.*) showing slight alteration.

Fig. 8. Senescent changes—initial stages of bands (*b.*), crescents (*k.*) are swollen.

Fig. 9. Senescent changes—bands (*b.*) strongly developed, crescents (*k.*) much altered.

Fig. 10. Senescent changes—bands (*b.*), discs (*d.*). Early stages in globule formation (*gg.*). Only outlines of crescents (*k.*) remain. Discs (*d.*) exhibit alteration.

Fig. 11. Senescent changes—further development of large globules (*gg.*). Bands (*b.*) less prominent, discs (*d.*) altered.

Fig. 12. Senescent changes—large globules (*gg.*) prevalent. Bands (*b.*) and discs (*d.*) tend to disappear.

Fig. 13. Senescent changes—remains of large globules (*gg.*). Discs (*d.*) in various stages of disappearance.

Fig. 14. Senescent changes—final stages in the disappearance of pectic substances, remains of bands (*b.*) and discs (*d.*).

PLATE XIII.

Figs. 15–19. *Series of diagrams illustrating the pectic changes which occur during senescence in Bramley's Seedling apples held in storage at laboratory temperature.*

Fig. 15. Cell showing two walls of contact (*w.*) and free wall surface (*f.*), outlines of crescents (*k.*), bands (*b.*), large globules (*gg.*), discs (*d.*).

Fig. 16. Thick transverse section showing the cells bounding an intercellular space (*s.*), free wall surfaces (*f.*), small globules (*g.*), connecting strands of pectic substance (*c.*).

Fig. 17. Transverse section—intercellular space (*s.*), free walls (*f.*). Swollen pectic substance at the re-entrant angles (*r.*). Pectic substance (*c.*) is observed connecting cells i and ii.

Fig. 18. Thick transverse section (December 3, 1925) showing the cells bounding an intercellular space (*s.*) with extensive development of small globules (*g.*) on the free walls (*f.*).

Fig. 19. Another section (November 30, 1923) taken from an apple obtained from the same locality as that used for Fig. 18 showing similar features.

Figs. 20–3. *Series illustrating advanced pectic changes in Bramley's Seedling apples as observed after ten months' storage at* 3° *C.*

Fig. 20. Thick section (August, 1924), low magnification, showing the general appearance of the tissues, intercellular spaces (*s.*), walls of contact (*w.*), free wall surfaces (*f.*).

Fig. 21. Single cell isolated from tissue illustrated in Fig. 20, high magnification, showing three walls of contact (*w.* i, *w.* ii, *w.* iii), altered discs (*d.*), remains of large globules (*gg.*), free wall (*f.*) showing a few small globules (*g.*).

Fig. 22. Group of cells (August 7, 1924) bounding an intercellular space (*s.*), walls of contact (*w.*); (*w.* i) showing large globules (*gg.*); (*w.* ii) and (*w.* iii) showing remains of globules (*gg.*) and bands (*b.*); free walls (*f.*).

Fig. 23. Thick section showing cells bounding an intercellular space (*s.*), free walls (*f.*) showing globules of various sizes.

Fig. 24. Groups of cells in an advanced state of senescence from the sub-epidermal region of the apple, showing cell separation in the various stages. (*α*) Cells still united. (*β*) Cells in various stages of separation.

PLATE XIV.

Fig. 25. Groups of completely separated cells showing plasmolysis of the cell contents and formation of bottle-shaped cells (*b.s.*).

Figs. 26–8. *Series illustrating the action of reagents on pectic substances.*

Fig. 26. Transverse section of young Bramley's Seedling apple (July 25, 1924) soaked in KOH after preliminary soaking in HCl. The intercellular spaces (*s.*) are filled with pectic substance (*p.s.*).

Fig. 27. Transverse section of young Bramley's Seedling apple (August 5, 1924) soaked in 5 per cent. HCl for three days, followed by 50 per cent. HCl one day, and 50 per cent. KOH five days. Masses of pectic substance (*p.*) in the intercellular spaces (*p.s.*) and in the cell-walls (*p.*).

Fig. 28. Transverse section of young Bramley's Seedling apple (June 16, 1924), soaked in HCl, then in KOH, and subsequently boiled in KOH (5 per cent.). The cells separate on the slightest pressure. Note complete absence of pectic substance.

Fig. 29. Transverse section of two cells from a Bismarck apple (April 24, 1924) stored at 1° C., showing natural phenomena comparable to those produced by reagents (cf. Figs. 26–8), namely, stain-

ing patches of irregular shape up to 28 × 40 μ in size situated in the walls of contact. These patches are presumably due to the alteration of the discs, which in the Bismarck apple are relatively large, reaching 16 μ in diameter. The remaining portion of each wall of contact is unstained.

Figs. 30–2. *Series illustrating the pectic changes occurring in Bramley's Seedling apples affected with internal break-down* (*November* 4, 1925) *held in storage at laboratory temperature.*

Fig. 30. Single cell showing three walls of contact (*w.*) almost depleted of pectic substance, and feebly staining free wall surface (*f.*) showing various stages in the disappearance of globules (*g.*).

Fig. 31. Single cell in which it is difficult to distinguish the walls of contact owing to a general depletion of the pectic substances. Walls of contact (*w.*) with altered discs (*d.*), free wall surface (*f.*) with globules (*g.*) in various stages of disappearance.

Fig. 32. Very advanced stage. The cells themselves are entirely depleted of pectic substance. Stringy masses of pectic substance (*p.*) are observed between the cells.

Figs. 33–4. *Pectic changes in Jonathan apples affected with internal browning.* Cells from the water-soaked and brown regions. Walls of contact (*w.*) showing little pectic substance; free wall surfaces (*f.*) with globules (*g.*) in various stages. Note that the middle lamella pectic substance is not dissolved.

Figs. 35–8. *Series illustrating the pectic disturbances produced by storage in an atmosphere of carbon dioxide for ten days at laboratory temperature.*

Fig. 35. Transverse section showing pectic substance (*p.*) at the re-entrant angles made by two cells in contact.

Fig. 36. Three separated cells showing connecting strands of pectic substance (*p.c.*) and irregular masses (*p.*) also of pectic nature.

Fig. 37. Single cell showing dome-shaped pectic masses (*p.*) probably arising from rupture of connecting strands.

Fig. 38. Stage in the formation of the stringy masses of pectic substance (*p.*) shown in Fig. 32.

Figs. 39–41. *Series illustrating the pectic changes resulting from a tissue lesion* (*Bramley's Seedling Apple*).

Fig. 39. Group of cells viewed from above. Intercellular space (*s.*); pectic substance (*p.*) uniting cells i, ii, and iii; globules (*g.*) on free walls (*f.*).

Fig. 40. Transverse section showing bands of pectic substance (*p.*) connecting the cells.

Fig. 41. Transverse section showing intercellular space (*s.*) with globules (*g.*) on the free walls (*f.*).

Figs. 42–6. *Series illustrating the pectic changes caused by fungal attack* (*Cytosporina ludibunda*).

Fig. 42. *Pectic changes in cells slightly in advance of the invading mycelium.* Intercellular space (*s.*), connecting strands of pectic substance (*p.s.*), discs (*d.*) in various stages of alteration, globules (*g.*) on the free walls (*f.*), walls of contact (*w.*).

Figs. 43–4. *Pectic changes in the tissues occupied by the invading mycelium.*

Fig. 43. Single cell showing walls of contact (*w.*) with remains of discs (*d.*). The pectic substance has almost disappeared from the free walls (*f.*).

Fig. 44. Thick section stained with cotton blue. The mycelium (*my.*) of the fungus surrounds the cells. Appressoria (*a.*), remains of pectic substance (*p.*).

Figs. 45–6. Cells from the infected region of the tissue stained with ruthenium red. Note that the appressoria (*a.*) are deeply stained, indicating the absorption of pectic substances by the fungus. Walls of contact (*w.*), free walls (*f.*), remains of pectic substance (*p.*).

Figs. 47–51. *Series illustrating the distribution of pectic substances in the pear.*

Fig. 47. Single cell from mature pear tissue showing walls of contact (*w.*), free walls (*f.*), discs (*d.*), suggestion of bands (*b.*).

Fig. 48. Cells from tissue in an advanced stage of ripeness, showing walls of contact (*w.*), discs (*d.*), small crescents (*k.*). The free walls (*f.*) present a granular appearance. Intercellular spaces (*s.*).

Fig. 49. Senescent changes—group of cells showing initial stages in cell separation. Connecting strands of pectic substance (*p.*), wall of contact (*w.*) showing altered discs (*d.*) and globules (*g.*).

Figs. 50–1. Similar groups of cells showing aggregations of pectic substances (*p.*) on the separated walls and formation of bottle-shaped cells (*b.s.*) and intercellular spaces (*s.*) in Fig. 51 filled with pectic substance (*p.s.*).

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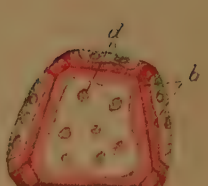

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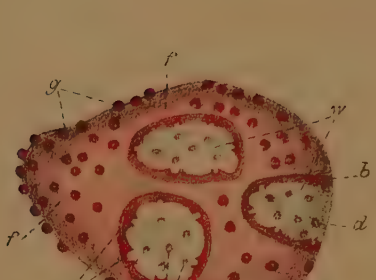

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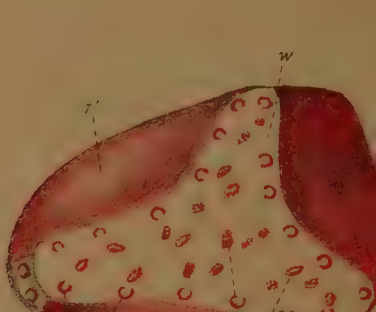

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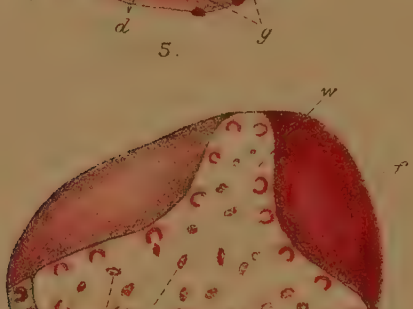

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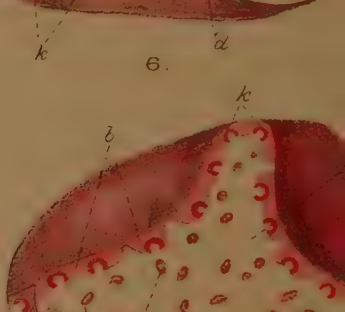

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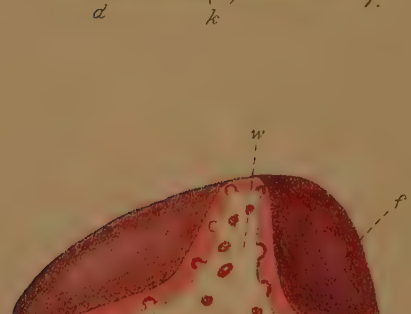

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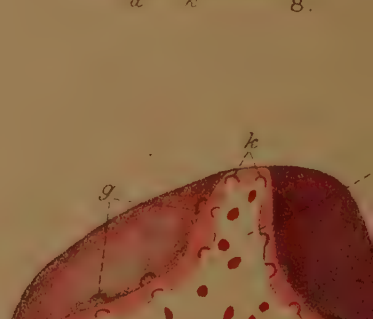
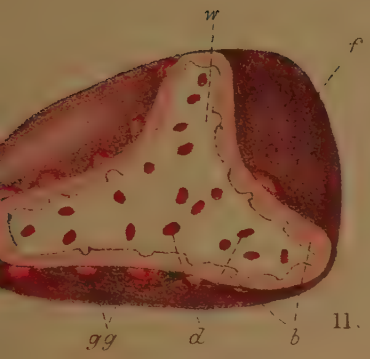


10.

CARRÉ & HORNE—PECTIC MATERIALS OF CELL WALL.



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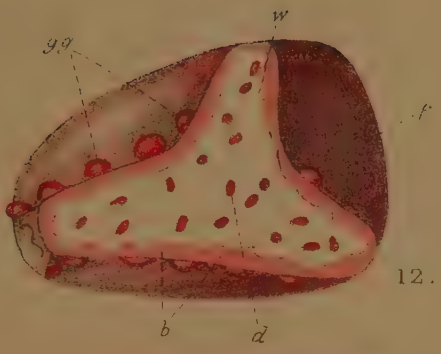

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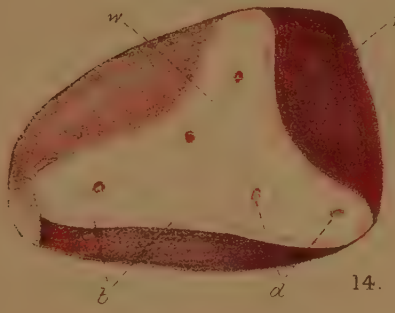

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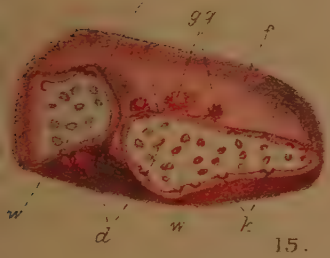

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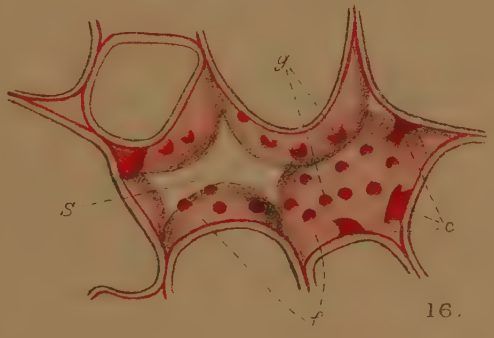

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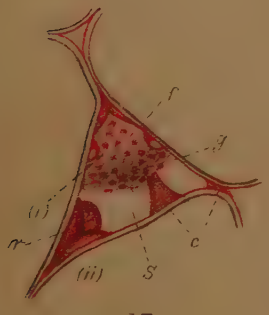

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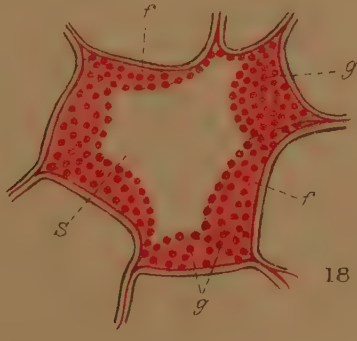

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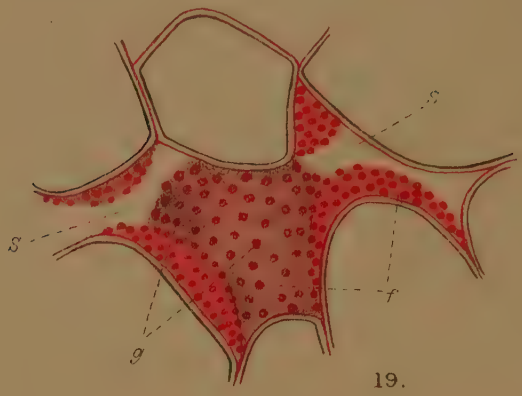

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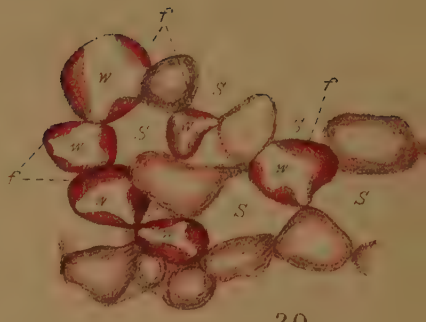

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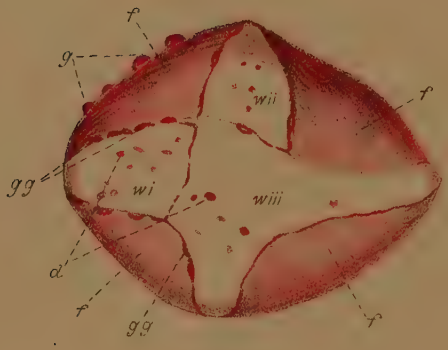

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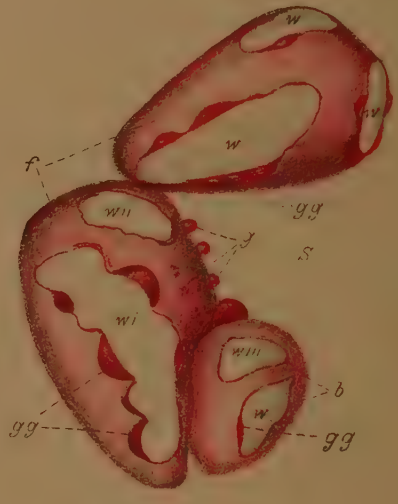

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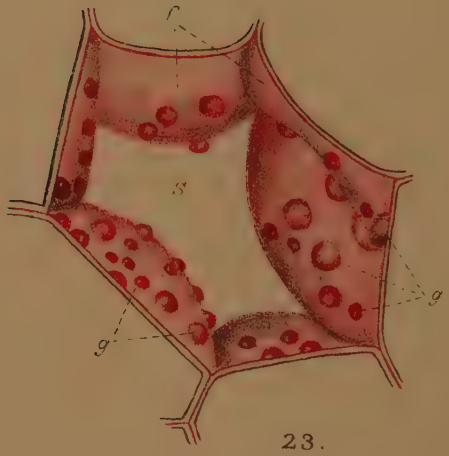

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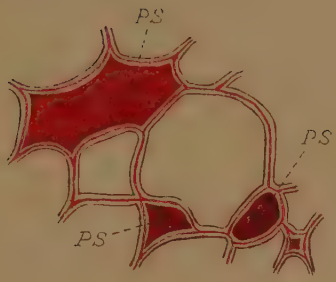

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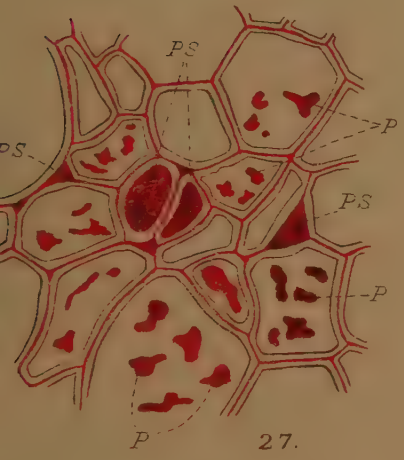

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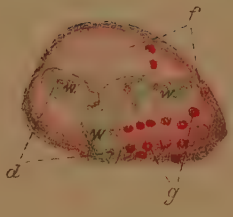

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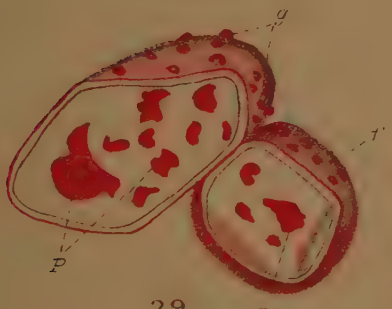

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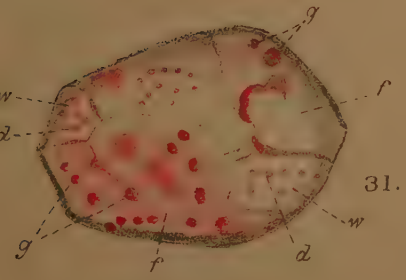

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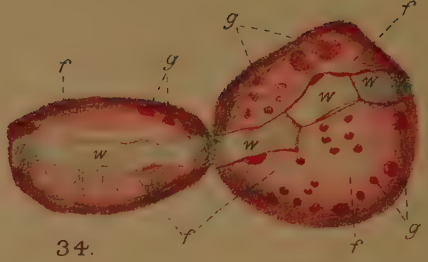

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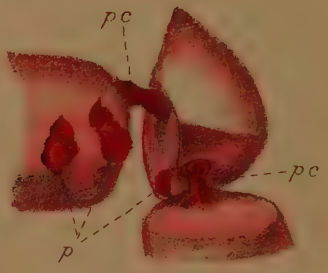

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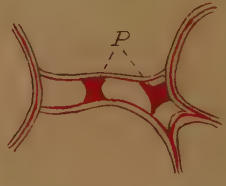

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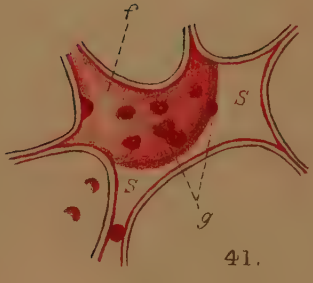

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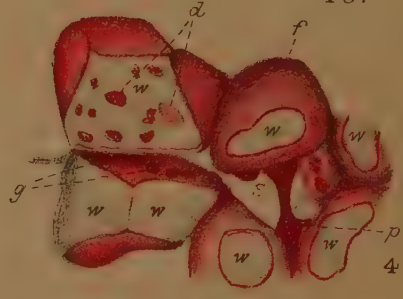

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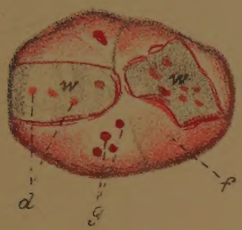

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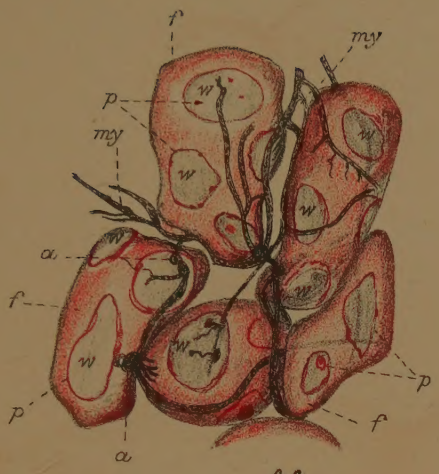

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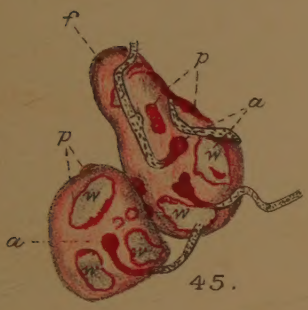

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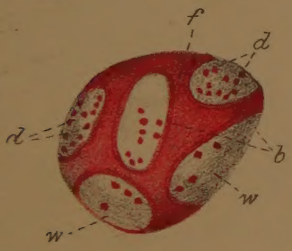

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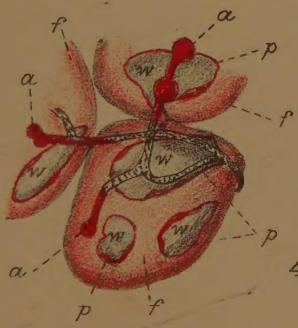

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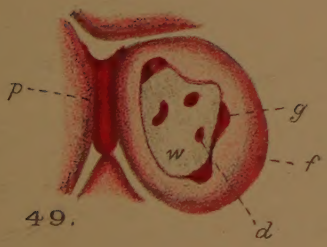

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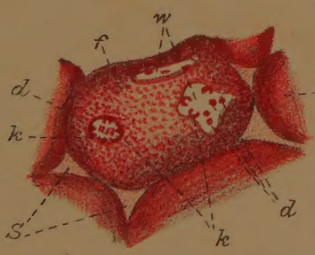

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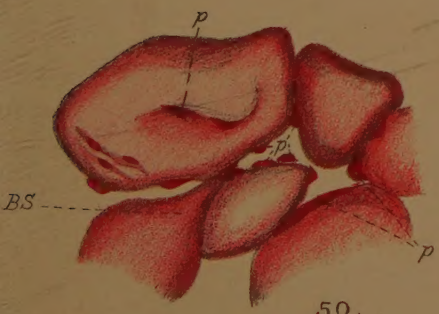

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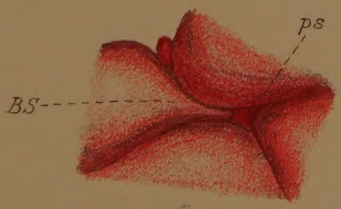

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